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PHYSICAL IMITATIONS OF THE ACTIVITIES OF AMŒBA.

H. S. JENNINGS.

PHYSICAL imitations of the activities of lower organisms, such as are given us by Bütschli and Rhumbler, have always taken a place among the "startling achievements" of science. They arouse a lively interest in the popular mind as well as in the minds of men who are seriously studying the problems which such activities present. Anything which promises a bridge from the inorganic to the organic, from the physical to the vital, demands attention. Almost all men have definite convictions as to the relation of these two fields, — convictions which are foundational for the whole superstructure of intellectual or religious life; anything which touches these convictions must awaken interest.

How far have the physical imitations of vital activities gone? What do they really show as to relation of physical and vital? In the present paper such physical imitations as relate to one of the lowest organisms, Amœba, will be examined with these questions in view. The greater number of the physical experiments relate directly to Amœba, attempting to imitate its behavior. The writer has recently made a thorough re-examination of the behavior of this animal, the results of which have been published elsewhere (Jennings, :04), so that opportunity is presented for a comparison between the imitations and the reality. By determining to what extent the physical imitations throw light on the behavior of Amœba, we shall perhaps have a fair measure of what has been accomplished in this way, and of the promise for the future.

What is the real purpose of the physical imitations of vital activities? Clearly, the final purpose is to show what physical factors are at work in these activities. But this end may be followed in many ways; what is the special purpose of the imitations?

In the best cases the physical imitations arise as follows: There is first a study of certain vital activities. This is followed by construction of a hypothesis as to the nature of the factors at work, — an explanation of the activities in terms of phenomena already known. The third step is to determine by experiment whether the supposed known factors can produce such activities; these factors are combined in appropriate ways and the results observed. If they bring about activities similar to those shown in the vital phenomena, then the explanation gains much in probability, and we have an "imitation" of the vital activities. What the imitation shows is then, as Rhumbler ('98, p. 108) has well said, that the factors assumed to be at work really can produce such activities as we attribute to them, — and this is a long step in advance. There still remains the question whether the factors in our imitation actually *are* those at work in the vital phenomena.

To enable us to judge intelligently on this final question we need an accurate knowledge of the phenomena to be explained and of the forces at work in the imitation, that they may be closely compared; imitations founded on external resemblance are likely to be misleading. We have indeed three factors to be compared, — the explanation as it exists in the mind of the investigator, the physical experiment, and the vital activity. In the best cases these three must agree; the explanation fits the experiment, and the experiment is essentially similar to the vital phenomenon, so that the explanation fits the latter also. But the explanation given may fit the physical experiment and not the vital activity, or it may not even fit the experiment; we shall find examples of both these cases.

In the commoner case, where the explanation given does fit the physical experiment, how are we to judge whether the vital activity is to be similarly explained? Evidently an explanation based on an imitation can at best fit the vital activity only in so far as the latter agrees with the imitation. Points in which it does not agree must be attributed to other factors, and if these points are essential ones for the explanation given, then we must conclude that the vital activity is not explicable in the way proposed. Further, we must determine whether certain conditions, preceding or following, which the explanation requires are actually fulfilled in the vital phenomena.

Imitations of the movements and of the variations in form have been oftenest attempted. Almost without exception the imitations are based on the hypothesis that these phenomena in Amœba are due to local changes in the surface tension of a fluid mass. Among the earliest experiments of this sort were those of Gad ('78). Gad placed drops of rancid oils (oils containing fatty acids) in weak solutions of alkali; for example, cod liver oil in 0.2 to 0.5 % sodium carbonate. As a result of the reaction between the fatty acid and the alkali soap is produced. This lowers the surface tension of the drop of oil here and there; as a result the drop changes form, sending out projections having an external resemblance to the pseudopodia of Amœba. A number of figures showing the forms taken by oil drops under these conditions are given in *Verworn's General Physiology.* Gad pointed out the resemblance of these forms to those shown by Amœba, but did not carry the matter farther.

Quincke ('79, '88) pursued further the study of movements caused in the manner just described, and put forth distinctly the view that the movements of Amœba (as well as of other protoplasmic masses) are due to similar causes. Quincke found that egg albumen might take the place of the sodium carbonate in the experiments above described; soap is then formed and movements occur as when the alkali is used. He held that Amœba is covered externally by a thin lamella of oil; that albuminous soaps are formed on the inner surface of this, thus decreasing the surface tension, and that the movements and changes of form are due to these changes in surface tension.

Most celebrated of all imitations of amœboid movements are those of Bütschli ('90, '92). Bütschli mixed slightly damp, powdered potassium carbonate with old olive oil, of a certain degree of rancidity, and brought drops of the mixture into water on a slide. (Directions in Bütschli, '90.) After standing twenty-four hours the drops are washed and new water or glycerine supplied. The drops now show streaming movements, send forth projections (see Fig. 1, *b*), and move about. The external resemblance to the phenomena shown in Amœba is very striking. The movements are caused as follows: The potassium carbonate is dissolved by the water and acts on the oil, forming soap. Thus after a time the oil drop is permeated throughout by minute globules of soapy water, forming a foam-like emulsion. At times one of these globules of soap bursts on the outside of the drop of oil; the soap then spreads over the surface of the oil, lowering its surface tension in the region affected. At once a projection is formed here, currents flow from within the drop toward the region of lowered tension, and the entire drop may move in that direction.

Bütschli held that the movements of Amœba take place in a similar manner. He considers that protoplasm has an emulsion structure similar in a general way to that of the oil drops, — though of course the constituents are not the same. At times the meshwork enclosing the globules breaks at the outer surface of the Amœba, allowing some of the enclosed fluid to spread over the surface. This lowers the surface tension, causing Amœba to move in the same manner as the drop of oil.

Bütschli is inclined to attach much significance to the fact that the oil drops which move in the way described have a foam-like emulsion structure, and to consider this as a support to his view that the similarly moving protoplasm is similarly constituted. But such movements are by no means specially characteristic of fluids having a foam-like or emulsion structure; many drops having this structure do not show the movements, while other drops which have not this structure show the movements equally well, as we shall see. The movements require only that there shall be some method of producing local changes in surface tension; this may be easily brought about without the emulsion structure.

Bernstein (:00) produced similar movements in drops of mercury. Sufficient mercury to make a drop or disk five to ten millimetres in diameter is placed in a flat-bottomed watch-glass. Over it is poured some 20% nitric acid, and thereto is added a quantity of a strong solution of potassium bichromate. The mixture acts chemically on the mercury, lowering its surface tension. The intensity of the action varies locally, so that the surface tension is decreased now here, now there. As a result the mercury moves and changes form in a striking manner, sending out projections or becoming wholly irregular, at the same time moving from place to place.[1]

The present author (:02) has given another method of observing such movements. A mixture of three parts glycerine and one part 95 % alcohol is placed on a slide and covered with a large cover-glass, supported near its ends by glass rods. Beneath the cover-glass a drop of clove oil is introduced by means of a medicine dropper drawn to a fine point. The alcohol acts locally on the surface of the clove oil, decreasing its surface tension here and there. As a result the clove oil drop changes form, sends out projections and moves from place in a striking manner. The phenomena shown are similar to those in Bütschli's drops of oil emulsion. The experiments are much easier to perform than those of Bütschli; by varying slightly the amount of alcohol in the mixture one can always be certain of getting marked results. But the movements do not continue so long as in Bütschli's experiments.

In all these experiments the movements are due to local changes in surface tension. When such a local change is produced on the surface of a fluid drop a characteristic set of currents results. From the region of least tension surface currents pass in all directions, while an interior current passes toward the region of least tension. The reason for these currents may be seen by imagining that the drop is covered with a stretched India rubber membrane in place of the surface film. If this stretched membrane is weakened or cut at a certain point the remainder of the membrane will pull away from this point, simulating the surface current. At the same time fluid from within will be pressed out at the weakened point,— thus simulating the central current toward the point of least tension.

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[1] The attempts of Herrera to imitate protoplasmic movements read almost like a travesty of those of the authors above mentioned. Herrera made a "synthetic protoplasm" by mixing together certain chemicals which analysis showed to exist in the protoplasm of one of the myxomycetes. This mixture contained "pepsine, ... peptone, acetic fibrine, oleic acid, soap, sugar, extract of bile, a considerable quantity of carbonate of soda, carbonates of calcium and ammonium, lactate of calcium, phosphates of calcium and magnesium, sulphates of calcium and iron, chloride of sodium, soap" (Herrera, '98, p. 118). When this miscellaneous conglomeration of chemicals was wet with water it showed, as one may well conceive, many diffusion currents. Herrera considers these as a "faithful reproduction of the internal novements of protoplasm described by Van Tieghem." In a later contribution Herrera ('98a) gives an imitation of amœboid motion based on the theory that Amœba is moved by the bubbles of carbon dioxide which it gives off in its respiration. Mix bicarbonate of soda with printer's ink so that a product is obtained having a sirupy consistency. Place on a surface wet with a weak solution of tartaric acid. Bubbles of carbon dioxide are produced, of course causing the mass to change form and move; the illusion of a living being is complete." It is only just to say that Herrera later gave up the idea that the movements of Amœba are caused in this manner.

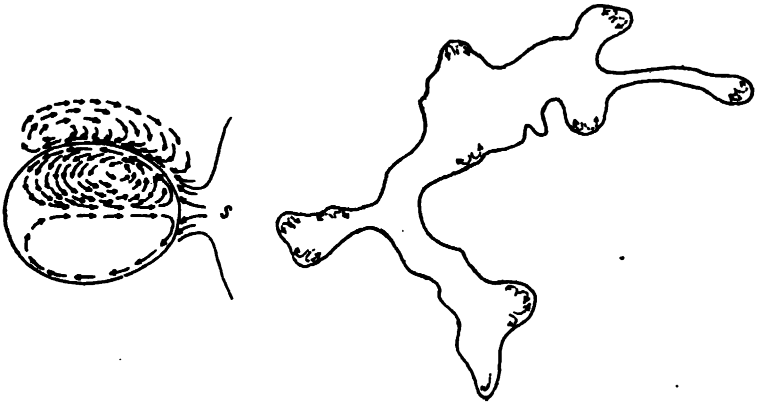

Fig. 1 *a*. Fig. 1 *b*.

Fig. 1.— Currents produced by local decrease of surface tension, after Bütschli. *a*, Currents in an oil drop when the surface tension is decreased at one end by contact with a soap solution (*s*); surface currrents away from the point of lowered tension; a central current toward this point. *b*, One of the drops of oil emulsion, showing the irregular form and the characteristic currents at the tip of each projection.

The characteristic currents may be seen in Bütschli's experiments or in those with the drops of clove oil, if some soot or India ink has been mixed with the oil. Such currents are represented in Fig. 1, taken from Bütschli. If the axial current carries forward more fluid than the superficial currents carry backward, the drop may elongate in the direction of the axial current and move as a whole in the same direction. This often occurs.

Such currents as are shown in Figure 1 are an invariable feature of movements of fluids due to local decrease in surface tension. Indeed, these currents are the characteristic phenomena; they may be the only movements that occur.

If, then, the movements of Amœba are really produced as they are in the imitations, by means of local changes in surface tension, we must expect to find in Amœba these characteristic currents. In an Amœba moving in a certain direction there should be a central current forward and superficial currents backward. In an extending pseudopodium the central current should be toward the point, the superficial currents away from it. Do such currents exist?

There is evidently a central current forward. But are the superficial currents backward, as the theory requires? In studying the movements from above, without the aid of experiment, it is difficult to determine this point. But there are certain appearances on the lower surface and at the lateral margins which give the impression that such backward currents may exist. In fact Bütschli, Rhumbler and others became convinced of the existence of such currents. The movements of Amœba were thus brought into full agreement with those of the drops moving as a result of local decrease in surface tension. This is brought out clearly by an examination of the figures of the currents in Amœba given by Bütschli and Rhumbler, copied in Figure 2. It was then almost inevitable to conclude that the same causes are at work in the two cases; that the movements of Amœba are due to local changes in surface tension.

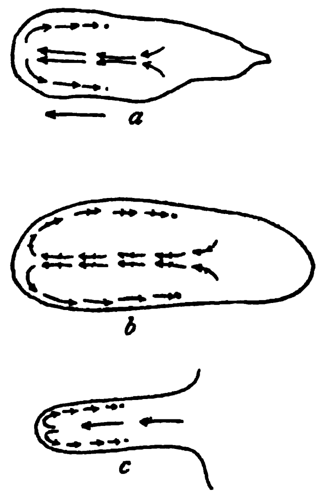

Fig. 2.— Diagram of currents in a moving Amœba, according to Bütschli and Rhumbler. *a*, Diagram of the currents as seen from above, after Rhumbler; *b*, diagram of the currents in side view, after Rhumbler; *c*, diagram of the currents in an advancing pseudopodium, after Bütschli.

In the extended experimental study of the activities of Amœba recently made by the present writer (:04), it was shown that the supposed backward currents of the surface do not exist. On the contrary, all parts of the surface which are not attached to the substratum are typically moving forward, in the same direction as the central current, while the attached parts of the surface are at rest. The movement of Amœba is thus of a rolling character; the upper surface continually passes around the anterior end to form the lower surface; this then remains quiet until it is taken up by the posterior end as the latter moves forward. The movements in an advancing Amœba are indicated in Figure 3. In a projecting pseudopodium the movements are of the same character as those at the anterior end (Fig. 3), save when the pseudopodium projects freely into

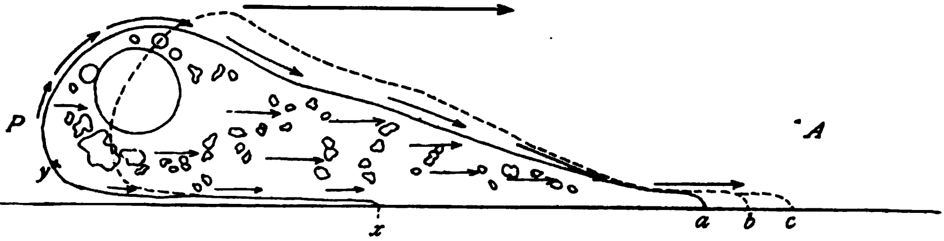

FIG. 3.— Diagram of the movements of an Amœba in locomotion, side view. The arrows show the direction of the currents; the longer arrows indicating more rapid movement. The large arrow above shows the direction of locomotion. The anterior end (A) is thin and attached to the substratum as far back as *x*; the lower surface from *a* to *x* is at rest. The posterior end (*P*) is high and rounded, and free from the substratum. *a*, *b*, *c*, successive positions occupied by the anterior end. The broken outline shows the position occupied by the Amœba a little later.

the water, being nowhere in contact with a solid. In the latter case the entire surface moves outward, in the same direction as the tip.

Details of the observations and experiments which demonstrate the movements to be of the character just set forth are given in an extensive paper published elsewhere (Jennings, :04). The movements were determined chiefly by observing the motion of objects attached to the outer surface of Amœba, of objects partly imbedded in the outer layer, and of particles within the body. The movements as thus studied are clear, and exclude the possibility of the typical existence of backward currents on the surface.

It appears then that Amœba does not move in the same manner as do the imitations based on local changes in the surface tension of a fluid mass. The currents which form the characteristic features in the latter case are not present in Amœba.

Neither theoretically nor practically does there appear to be any evidence that movements due to changes in surface tension can take place without these characteristic currents. We cannot then consider the movements of Amœba to be due to a decrease in surface tension at the anterior end, as in the "imitations." In precisely the feature which led to the supposition that the movements in the two cases were of the same character we find that there is actually an absolute contrast. In Amœba the surface currents are in the direction of movement of the mass, and in the same direction as the central current; in the imitations they are in the opposite direction.

Clearly the surface tension theory will not account for the phenomena as they actually exist. This becomes still more evident when we consider the formation of pseudopodia not in contact. In these there is not only no backward current, but also no resting surface; axis and surface move outward in the same direction as the tip. Such movements are not producible by local changes in surface tension. The "imitations" are imitations only to the extent that they are fluids and that they move; they are not imitations so far as the nature of the movements and their cause is concerned.

A much more nearly accurate imitation of the movements of Amœba may be produced with gravity as the active agent in place of surface tension. A drop of water moving down hill on a surface to which it does not cling strongly shows the same rolling movement that we find in Amœba. The lower surface (in contact with the substratum) is at rest, while the upper surface moves forward and passes continually around the anterior end to the lower surface. But we know that gravity is not the active agent in the movement of Amœba.

An imitation of the usual locomotion of Amœba that is accurate even to minute details is described by the present author in the paper on the behavior of Amœba already cited (Jennings :04). A drop of fluid resting on a substratum is caused to adhere to the substratum more strongly at one edge than at the other. Thereupon the drop moves toward the more adherent edge, and in so doing it shows exactly the form and movements of an Amœba in locomotion. The experiments may best be performed as follows: A piece of smooth cardboard, such as the Bristol board used for drawing, is placed in the bottom of a flat dish and on a certain spot on the cardboard is placed a drop of water. The whole is then covered with bone oil. This soaks into the cardboard, except where the latter is protected by the drop of water. After the board is well soaked in oil the drop of water is removed, leaving the whole surface covered with oil some millimeters deep. Now a drop of water or glycerine, to which has been added some fine soot, is placed on the cardboard under the oil. This drop is allowed to come in contact by one edge with the area which had been protected from the oil. To this area it adheres, the edge in contact spreads out as a thin sheet, and the rest of the drop is pulled over to the area. Its movement is then exactly that typical for a flowing Amœba, so that Figure 3 would do equally well for a diagram of the movements of such a drop as for those of Amœba. The resemblance extends to minute details; many of these are set forth in the author's paper above cited (:04). Among other things, the formation of pseudopodia in contact with the substratum may be imitated by making the area to which the drop adheres at one edge very small; then a projection is formed merely of the width of this area.

But this imitation, like the others, fails when we take into consideration the formation of pseudopodia which are nowhere in contact with a solid. Projections corresponding to these cannot be formed in the physical experiments just described, for in these adherence to a solid is the essential point. Since the entire anterior end of the Amœba can be pushed out into the free water, we find that Amœba can perform all the active operations concerned in locomotion without adherence to a solid. This effectually blocks any attempt to explain the movements of Amœba as due, like those of the drops in the experiments just described, to one-sided adherence to the substratum.

Thus none of the physical imitations gives us a clue to the physical agent actually at work in the movements of Amœba. The experiments last described are perhaps useful in giving us an idea of the direction of action of the forces at work in producing locomotion. Not even so much as this can be said of the surface tension experiments; the direction of action of the forces in these is evidently different from that in Amœba.

We may then turn to imitations of other activities of Amœba. Many attempts have been made to imitate certain of the reactions to stimuli — particularly the positive reaction to chemicals. Such imitations depend on the fact that a local decrease in the surface tension of a drop of fluid may be caused by contact with a chemical; the drop then moves in the direction of lowered tension. Some of the experiments based on this are the following:

Rhumbler ('99, p. 585) placed a small drop (60 to 90 μ in diameter) of castor oil in alcohol, and brought close to it the open end of a capillary tube containing clove oil, chloroform, or 5 % potassium hydroxide. The substance within the tube diffused out against the drop of castor oil and decreased its surface tension in the region of contact. Thereupon the usual currents were produced (Fig. 1), and the drop moved in the direction of lowered tension, finally entering the tube.

Bernstein (: 00) placed a drop of mercury in twenty per cent. nitric acid, then brought near it a crystal of potassium bichromate. By the chemical action the surface tension on the side of the drop next to the crystal is decreased. Thereupon the drop moves rapidly over to the crystal, and may push it about from place to place.

In the drops of clove oil in a mixture of glycerine and alcohol, described above (p. 8), similar movements may be caused (Jennings : 02). With a capillary pipette a little alcohol is brought near one side of the drop. This decreases the surface tension of the part affected; thereupon a projection is sent out toward the alcohol, and the drop as a whole moves toward it. If the drop is heated at one edge, by touching the cover glass near it with a hot wire, the clove oil moves toward the heated side, and may be induced to follow the wire for some distance.

In all these experiments the movement is due to local alterations in surface tension; the drop moves toward the region of lowest tension; there is a central current in the direction of locomotion, and surface currents in the opposite direction. In Amœba, on the other hand, as we have seen, the movements cannot be considered due to local decrease in surface tension. There are no superficial currents away from the region toward which the animal moves, but all parts that are in motion move toward the object causing the reaction. (For details, see Jennings, :04.) The experiments do not imitate the essential features of the action of Amœba, and do not show us the causes at work in its behavior. The reactions of Amœba are not simple direct results of the physical action of the agents producing them, but are indirect, like those of higher animals.

Many imitations have been devised for the taking of food by Amœba. Rhumbler ('98) holds that the ingestion of food by Amœba is due to physical adhesion between the liquid protoplasm and the solid food. He shows that drops of all sorts of fluids take in certain solids in this manner. A drop of water placed at the edge of a plate of glass draws to itself and envelopes splinters of wood and various other solids which come in contact with it. Glycerine, oils, white of egg, gum arabic, mastax varnish, etc., are shown to do the same. A convenient way of showing this is to fill a capillary glass tube with the fluid, then to bring a small piece of the solid in contact with the free surface of the liquid at the end of the tube. The pulling of the solid into the liquid is due to the adhesion of the two, in connection with the surface tension of the liquid.

These experiments of Rhumbler show that food might be taken in this manner, not that it is so taken. Careful study shows that there is in most species of Amœba no adhesion between the protoplasm and the food body. Food is taken by actively enclosing it along with a small quantity of water; the fact that no adhesion exists between it and the protoplasm is strikingly evident, and occasions much difficulty in the ingestion of food. (For details, see Jennings, :04, and compare the similar account of food-taking by Le Dantec, '94.) Thus the experiments do not really imitate the essential features of the behavior in Amœba. Only in Amœba verrucosa and its close relatives is there evidence of adhesion between the animal and its food. But even here there is adhesion equally to bodies which do not serve as food and are not ingested, so that for the ingestion itself an additional factor is necessary.

One of Rhumbler's most striking experiments is an imitation of the method by which Amœba takes as food a long filament of Oscillaria, coiling it up and enclosing it. The Amœba settles down somewhere along the filament, lengthens out upon it, and bends it over, forming a loop. This process is repeated until the long filament forms a close coil within the Amœba (figures in Rhumbler, 1898, p. 211, Lang, :01, p. 39; a similar account with figure in Leidy, '79, p. 86). Rhumbler considers this remarkable process to be brought about as follows: The Amœba adheres to the filament. It lengthens out along it, just as a drop of water lengthens out along a filament to which it adheres. Owing to the surface tension of the fluid protoplasm, impelling it to take the spherical form, it pulls on the two halves of the filament, producing a thrust inward from both directions. Gradually the enclosed parts of the filament are softened in the digestive processes of the Amœba. The softened portion then yields to the thrust from both directions and bends, so that more of the filament can be pulled into the Amœba by the tension of its surface film. The Amœba then lengthens out farther, owing to adhesion; more of the filament is softened and yields farther, so that more is pulled in by surface tension. This process continues until the filament is completely coiled up and enclosed.

On the basis of this explanation Rhumbler devised an imitation of the process. A chloroform drop is placed in the bottom of a watch-glass of water. A long fine thread of shellac, obtained by heating two pieces of shellac in contact over a flame and rapidly pulling them apart, is brought in contact with the drop. The latter envelopes the filament in some portion of its length, then proceeds to coil it up, as Amœba does with the Oscillaria filament; after a time the shellac thread is completely enclosed within the chloroform drop. The mechanism of the process is conceived to be the same as that above given for Amœba and the Alga filament.

This experiment is an interesting example of one of the numerous difficulties which beset the worker along such lines, — of the fact, namely, that even the imitation may not agree with the explanation given. The coiling up of the shellac thread by the chloroform is not explicable in the manner supposed by Rhumbler; the surface tension of the drop has really nothing to do with it. This is shown by the fact that such a thread of shellac is coiled up in exactly the same manner if submerged in a large vessel of chloroform, so that it is nowhere in contact with the surface film. The coiling up is apparently due to strains within the shellac filament, produced when it was pulled out, and to the adhesiveness of its surface when wet with chloroform. There are no corresponding factors in the Oscillaria thread; this will indeed, as Rhumbler has shown, straighten out again when released by the Amœba. The process by which Amœba coils up the Oscillaria filament must thus be of an essentially different character from that occurring in the experiment. The explanation given by Rhumbler may of course still be correct for the process in Amœba, though it is not correct for his imitation of the process.

Amœba does not ingest every small object with which it comes in contact, but exercises an evident choice as to the substances which it takes as food. Physical explanations and imitations of such choice have been given. We may notice especially those set forth by the present author (:02) in extension of certain experiments of Rhumbler. A drop of chloroform is placed in the bottom of a watch-glass of water, and with fine tweezers pieces of various substances are brought in contact with its surface. Some are at once taken in; others are not, or are thrown out if forced into the drop. Glass, sand, dirt, wood, gum Arabic, and chlorate of potash are rejected; shellac, paraffin, styrax, and hard Canada balsam are accepted. The selection or rejection depends upon the relative amount of adhesion between the solid object on the one hand and the chloroform and water on the other. Those which adhere more strongly to the chloroform than to the water are taken in; others are rejected.

These experiments show how choice might occur in an organism; they do not show how it actually occurs in Amœba. Food-taking is usually, as we have seen, not accompanied by adhesion between Amœba and the food, so that choice of food cannot be explained as due to the fact that some substances adhere while others do not.

Rhumbler ('98) has given a physical imitation of the taking in of a food body and of later giving off the undigested residue (defecation). A rod of glass covered with a thin layer of shellac is taken in by a drop of chloroform (as a result of adhesion). The shellac is dissolved off by the chloroform and the glass rod is then thrown out, since the chloroform does not adhere to it. This imitation, like the others, loses much of its force in view of the fact that food-taking is not usually due to adhesion and that substances which do not adhere are taken as food; defecation cannot then be explained as due simply to lack of adhesion.

In all the imitations thus far we find that the physical factors at work cannot be considered the same as those that are acting in Amœba. The imitations are such only in purely external features. There exist certain imitations, however, in which this has not been proved to be the case. Thus, Rhumbler ('98) found that when chloroform drops are placed in water, the water gradually passes into the chloroform, collecting in minute globules, which later gather in a larger drop. This larger drop is finally given off to the outside. This process Rhumbler considers analogous to the formation and discharge of the contractile vacuole in Amœba. The present author (:04) has described imitations of certain movements of the pseudopodia in Amœba, produced in liquids partly covered with a solid layer; these are hardly of sufficient general interest to be detailed here. The most striking experiments which can still be considered with some degree of probability to indicate the factors really at work in certain processes occurring in the Rhizopoda are undoubtedly Rhumbler's imitations of the production of Difflugia shells. Since these deal with an organism closely related to Amœba, they may be described here.

The experiments may be performed as follows: Chloroform is rubbed up with fragments of glass in a mortar until the glass is reduced to the finest dust. Then with a pipette drawn out to a small.point drops of this mixture of chloroform and glass are injected into water. At once the grains of glass come to the surface of the drops so formed and arrange themselves in a single layer, without chinks or crevices, exactly as in the shell of Difflugia. The chloroform drop is thus covered with a shell having a striking resemblance to that of Difflugia. In place of chloroform, linseed oil or other oils may be used. The drops must then be injected into 70% alcohol, since the oil would float on water.

The factors at work in the formation of the "artificial shells" are diffusion currents within the chloroform, the adhesion of the bits of glass to its surface, and the action of surface tension in arranging and fitting together the bits of glass. Studies of the process by which the shell of Difflugia is formed at the time of division of the animal seem to indicate that the same factors may be at work in the living organism. (See Rhumbler, '98, p. 289.)

Reviewing our results, we find that few of the experimental imitations of the activities of Amœba stand before a critical comparison with what actually takes place in the animal. Such comparison shows in almost every case that the factors at work in the imitations are essentially different from those acting in Amœba. In particular, almost all the imitations based on local changes in surface tension break down completely.

What are we to conclude from this fact as to the part played by surface tension in vital phenomena? The tendency has been of late to attribute more and more of a rôle among life processes to surface tension. Amœba has been the chief place where the important part played by surface tension seemed really demonstrable; the movements, the reactions to stimuli, the taking of food, and the choice of food, were all attributed to this and closely related factors. With the demonstration of the complete failure of surface tension to account for the phenomena that were chiefly relied on to prove its importance, the supposition that it plays an immensely important rôle in life processes loses much of its weight. Surface tension may of course, in a more refined way than was supposed for Amœba, still play the large rôle in vital phenomena that some attribute to it. In the meshes of Bütschli's protoplasmic meshwork, or in the muscle fibrillæ (Bernstein), it may perhaps do what is demanded of it. Possibly the study of surface tension is still the most promising field for detection of the physical factors underlying life processes. But the surface tension theory must come to us shorn of the trophies of its prowess, — its supposed full explanation of most of the activities of Amœba, — and bearing instead the record of a complete defeat.

What positive results of value have the physical imitations of vital activities in Amœba to show? As we have seen, there are still two or three of these that may really give us a clue to the factors at work in the vital processes; at least this has not yet been disproved. Beyond this the positive results are of a very general character. The imitations show that a drop of fluid might, through physical factors, show locomotion, move toward certain agents and away from others, and exhibit choice in the taking in of certain substances and the rejection of others. But they do not show specifically through what physical factors the activities are as a matter of fact brought about in Amœba or any other particular organism.

The chief value of most of the attempted physical imitations is that of a trial. The method of trial and error is a method of progress in science as elsewhere. In these imitations a definite explanation of the phenomena is put on trial. The "trial" consists in a more careful study of the phenomena in question; it is as an inspiration to such study that the imitations are of great value. If as a result the explanation given is recognized as "error," that is in itself an advance; this particular trial will not need to be made again. Continued application of this method of trial and error must result finally either in the discovery of the real factors at work, or in the recognition that we are dealing with a new class of factors not found in physics.

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BIBLIOGRAPHY.

Bernstein, J.

1900. Chemotropische Bewegungen eines Quicksilbertropfens. Arch. f. d. ges. Physiol., Bd. 80, pp. 628–637.

Bütschli, O.

'90. Experimental Imitations of Protoplasmic Movement. Quart. Journ. Micr. Sci., vol. 31, pp. 99–103.

'92. Untersuchungen über mikroskopische Schäume und das Protoplasma. Leipzig. 234 pp., 6 plates.

GAD, JOHANNES.

'78. Zur Lehre von der Fettresorption. DuBois Reymond's Arch. f. d. Physiol., Jhrg. 1878. pp. 181–205. Taf. 2.

HERRERA, A. L.

'98. Sur le protoplasma synthétique et la force vitale. Bull. Soc. Zool. France, vol. 23, pp. 118–120.

'98a. Sur la manière de produire certains mouvements amiboides par un dégagement d'acide carbonique. Bull. Soc. Zool. France, vol. 23, pp. 128–130.

JENNINGS, H. S.

:02. Artificial Imitations of Protoplasmic Activities and Methods of demonstrating them. Journ. Appl. Micr. a. Lab. Methods, vol. 5, pp. 1597–1602.

:04. The Movements and Reactions of Amœba. Contributions to the Study of the Behavior of the Lower Organisms, No. 6. Carnegie Institution of Washington, D. C., Publication No. 16.

LANG, A.

:01. Lehrbuch der vergleichenden Anatomie der wirbellosen Thiere. Zweite Auflage, zweite Lieferung: Protozoa. Jena. 311 pp.

LE DANTEC, F.

'94. Études biologiques comparatives sur les Rhizopodes lobés et réticulés d'eau douce. Bull. Scient. de la France et de la Belgique, T. 26, pp. 56–99.

LEIDY, J.

'79. Fresh-water Rhizopods of North America. Rep. U. S. Geol. Survey of the Territories, vol. 12, 234 pp., 48 pl.

QUINCKE, G.

'79. Ueber Emulsionsbildung und den Einfluss der Galle bei der Verdauung. Arch. f. d. ges. Physiol. Bd. 19, pp. 129–144.

'80. Ueber periodische Ausbreitung von Flüssigkeitsoberflächen und dadurch hervorgerufene Bewegungserscheinungen. Sitz. ber. d. kgl. preuss. Akad. d. wiss. z. Berlin, Bd. 34, pp. 791–804.

RHUMBLER, L.

'98. Physikalische Analyse von Bebenserscheinungen der Zelle. I. Bewegung, Nahrungsaufnahme, Defäkation, Vacuolen-Pulsation und Gehäusebau bei lobosen Rhizopoden. Arch. f. Entw.-Mech., Bd. 7, pp. 103–350.

'99. Allgemeine Zellmechanik. Merkel und Bonnet's Ergebnisse der Anatomie und Entwicklungsgeschichte, Bd. 8, pp. 543–625.

nnings, H. S. Compliments of the author

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THE PSYCHOLOGY

OF A

PROTOZOAN

H. S. JENNINGS

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THE PSYCHOLOGY OF A PROTOZOAN.

By H. S. JENNINGS.

The nature of the psychic activities of unicellular organisms has of late become the object of considerable interest, though little work dealing with the problems in a fundamental way has been published since the researches of Verworn. The writer has recently made a perhaps more thorough study of the life activities of a typical infusorian, *Paramecium*, than has ever been made heretofore of any unicellular organism; the results of this study have been published in a number of papers in physiological journals.[1] This work was not done primarily from the psychological standpoint, and the papers referred to give nowhere a full and connected account of the bearings of these studies upon the psychological problems presented by the behaviour of the Paramecia. Yet taken together they enable an almost complete presentation to be given of the psychology of this animal; while there is reason to believe that Paramecium is in this matter typical of nearly or quite the whole class to which it belongs. In the present paper an attempt is made to bring together succinctly the observations which bear upon the psychic powers of this organism, in such a way as to present a complete outline of its psychology.

Paramecium is well known in every biological laboratory, living by thousands in pond water containing decaying vegetable matter. It is a somewhat cigar-shaped creature, about one-fifth of a millimeter in length, plainly visible to the naked eye as an elongated whitish speck. The entire surface of the animal is covered with cilia, by means of which it is in almost constant motion.

Now what are the phenomena in the life of Paramecium which require explanation from a psychological standpoint?

Examination shows that under normal conditions Paramecia are usually engaged in feeding upon the masses of Bacteria which form a thick zoogloea on the surface of the water in which they are found. These Bacteria form almost or quite their entire food. A first question then might be: How do they choose their food, selecting Bacteria in preference to something else?

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[1] Studies on Reactions to Stimuli in Unicellular Organisms. I. Reactions to Chemical, Osmotic, and Mechanical Stimuli in the Ciliate Infusoria. *Journal of Physiology*, May, 1897, Vol. XXI, pp. 258-322. II. The Mechanism of the Motor Reactions of Paramecium. *American Journal of Physiology*, May, 1899, Vol. II, pp. 311-341. III. Reactions to Localized Stimuli in Spirostomum and Stentor. *American Naturalist*, May, 1899, Vol. XXXIII, pp. 373-389. IV. Laws of Chemotaxis in Paramecium. *American Journal of Physiology*, May, 1899, Vol. II, pp. 355-379.

If Paramecia are placed on an ordinary slide such as is used for examining objects with the microscope, together with a small bit of bacterial zoogloea, and the whole covered with a cover-glass, it will soon be found that almost all the Paramecia, which were at first scattered throughout the preparation, have gathered closely about the mass of zoogloea and are feeding upon it. It will be seen even, that many Paramecia which cannot on account of the crowd get near enough to the mass to touch it are pushing close and shoving their more fortunate brethren, all apparently trying to get as near to the delicacy as possible. Some may be ten times their own length from the mass, but nevertheless crowd in from behind, apparently with the greatest eagerness. Here we have a related problem. How do the Paramecia collect thus from a distance about the mass? And what is the psychology of their crowding together thus, like a human crowd about a circus door? In the human crowd somewhat complex psychological qualities are involved; must we say the same for the Paramecia?

If we mount the Paramecia in the manner above described, but without the mass of bacterial zoogloea, we shall soon notice another phenomenon reminding us of human beings under like conditions. The Paramecia do not remain scattered as at first, but soon begin to collect into assemblages in one or more regions. It appears as if they did not enjoy being alone and had passed the word along to gather and hold a mass meeting in some part of the preparation; at least we soon find them nearly all in a little area near one end of the slide, with perhaps another smaller crowd off near the other end, while all the rest of the space is empty. Sometimes such a crowd becomes very dense; the Paramecia jam each other after the most approved human fashion, crowding as if all were trying to get near some popular orator in the center. If we watch such an assembly for some time, we find that the interest is apparently gradually lost; the Paramecia begin to separate a little,—not leaving the crowd entirely, but extending the area and wandering about its edges. The assembly thus becomes more and more scattered, the area in which the Paramecia swim back and forth being continually enlarged; but a rather sharp boundary is nevertheless maintained on all sides, as if by common consent no Paramecium was to pass farther out than all the rest.

Here we have what seems a decidedly complex psychological problem,—the beginning, or perhaps even a high development, of social conditions. In the culture jars, also, we find the Paramecia gathered into swarms, and any proposed psychology of the Protozoa must account for these social phenomena.

Further, we find that Paramecia seem to have decided preferences in taste. They have a special predilection for sour, gathering with apparent eagerness into a drop of any solution having a weakly acid reaction, while their pet antipathy is toward anything alkaline in character. A drop of fluid having an alkaline reaction is therefore left severely alone and remains entirely empty when introduced into a slide of Paramecia. They also seem to show decided preferences as to heat and cold; they collect in regions having a certain temperature, leaving a colder or warmer area to gather in such an optimum region, just as human beings do. The whole question of how animals are attracted by certain influences and repelled by others is one of the most fundamental problems to be solved.

Thus the ordinary daily life of a Paramecium seems, on the face of it, to present many complex psychological problems. Apparently they feel heat and cold and govern themselves accordingly, have decided preferences as to the nature of the substances dissolved in the water, seeking some, fleeing from others; they live upon one definite sort of food and find ways of discovering a mass of such food even when scattered at a distance from it, and finally, they are social, being commonly found in swarms together and finding means of getting together even when scattered over a wide area.

From observations of this sort, some authors have concluded that such animals have a complex psychology, lacking few of the factors to be distinguished in the psychology of the higher animals. Thus, Binet says, in the preface to his book on *The Psychic Life of Micro-organisms* "We could if necessary, take every single one of the psychical faculties which M. Romanes reserves for animals more or less advanced in the zoölogical scale, and show that the *greater part* of these faculties belong equally to micro-organisms." Thus, it could be maintained from the brief summary I have given of the activities of Paramecium that these animals have *sensations* of various sorts, since they distinguish heat and cold, acids and alkalies; that they exercise *choice* in that they gather in the regions of certain agents, while they turn away from others; that such choice in itself implies *intelligence*; that the choosing and gathering about masses of food implies a *memory* of the qualities of this substance as compared with others; that they show such *emotions* as fear by fleeing from injurious substances (Binet expressly states this); that finally, acute senses, memory, choice, social instinct, intelligence, and a whole host of higher mental attributes, are necessarily implied in the phenomenon of their seeking each other's society and gathering together even from a considerable distance into crowds.

Is it possible by a closer analysis of the phenomena to simplify this complex psychology which seems forced upon us by the observed facts?

First, we should examine a little more closely the structure of the animal to see what is here available for the production of these results. Often function depends upon structure to such an extent that what appears to be a complex activity is found to be only the automatic result of the simplest movements of a peculiarly constructed organ or set of organs.

Paramecium is an elongated animal, with one end (the anterior) narrower and blunter, while the other (the posterior) is broader and pointed. On one side of the animal (the oral side) a broad oblique depression, called the oral groove, runs from the anterior end to the mouth, in the middle of the body. Near the opposite side (the aboral side) are two contractile vacuoles imbedded in the protoplasm. The mouth is a small opening at the end of the oral groove in the middle of the body; from it a narrow ciliated tube, the gullet, passes into the internal protoplasm. In the center of the animal are imbedded the single large macro-nucleus and the single small micro-nucleus. The entire body is thus a single cell. Under ordinary conditions all the cilia of the body strike backward, which of course drives the animal forward. The stroke of the cilia is apparently somewhat oblique, for as the animal moves forward, it at the same time continually revolves on its long axis: in this way the oral and aboral sides continually interchange positions.

Now the structure and ordinary movements of the animal explain a certain activity which in higher forms may be associated with some degree of psychological complication, namely, the taking of food. Since the oral groove is ciliated like the rest of the body, when the cilia strike backward in the ordinary forward motion a current of water is produced running in the oral groove backward to the mouth. Small particles such as Bacteria, are thus carried automatically to the mouth. The mouth and gullet are ciliated and the cilia strike toward the interior of the animal, hence the particles arriving at the mouth are carried by the cilia into the interior, where they undergo digestion. The taking of food is thus purely automatic.

Moreover, as has long been known, Paramecia and similar animals seem not to exercise a choice as to the nature of the food which they take. Any small particles such as will pass readily down the gullet are swallowed with the same avidity as the Bacteria, it matters not how indigestible they may be.

But, as we have seen above, if a small piece of bacterial zoogloea on which the animals feed is introduced into a preparation of Paramecia, the latter soon find it and crowd around it. It seems possible, therefore, that the choice of food takes place merely a step sooner than with higher animals, the Paramecia choosing the food by gathering around it,—then taking whatever comes. To test this we introduce a bit of filter paper into the preparation in place of the bacterial mass. The Paramecia collect about it exactly as about the zoogloea. They gather from all parts of the preparation and crowd upon it with the same apparent eagerness as previously upon the food mass. The same results are gained with bits of cloth, cotton, sponge, or any other loose or fibrous bodies. The Paramecia remain assembled about such bodies indefinitely, the oral cilia working away at bringing a current to the mouth, which current carries no food particles whatever.

Thus it appears that Paramecia exercise no choice as to the nature of the substances which they use for food, gathering indifferently about loose fibrous bodies of any sort, and swallowing particles of any kind or none at all, as chance may direct. We may cut out, therefore, any psychological qualities deduced alone from the supposed choice of food, putting in their place merely the fact that Paramecia react in a peculiar way when they come in contact with bodies of a certain physical texture. The reaction consists essentially of a quieting of the cilia over the greater part of the body, while those in the oral groove continue to strike backward, causing a current toward the mouth,—the body of the animal remaining nearly or quite at rest. It is important to recognize, in calling this a reaction, that it is not shown by a movement, but by a *cessation* of part of the usual motion.

Having been so successful in reducing the matter of feeding to simple factors, we may attack at once the most complex problem of all—the social phenomena shown in the gathering together of the scattered animals into a close group, as already described. Is there any way of dispensing with the sharpened senses, memory, social instinct, intelligence, and the like, which seem to be involved in these phenomena?

The possibility suggested itself that these collections might be due to the presence of some substance which was attractive to the Paramecia, and into which all would gather with one accord,—so that the fact that they approached each other would be a secondary result. This led to an extended study of the chemotaxis of Paramecia, the results of which are detailed in the first of the papers above referred to. It was found that Paramecia are attracted by all acids, and that in the case of any unknown substance having marked attractive properties, it can be predicted with a high degree of certainty that this substance will be found to have an acid reaction. Carbonic acid (CO_2) especially was found to exercise a strong attraction on the infusoria.

Now these animals, like all others, of course excrete carbon dioxide, which must therefore find its way into the water. The quantity of CO_2 thus produced by one of the dense assemblages of Paramecia was shown to be distinctly appreciable by chemical reagents, by means of the following experiment: Paramecia were mounted in water to which a distinctly reddish color was given by mixing with it a small quantity of rosol. This substance has the property of being decolorized by carbon dioxide. The rosol does not injure the Paramecia, and they soon gather together in a dense collection, as in ordinary water. By observing the slide against a white background it is soon noticed that the solution is losing its color about the group of Paramecia. The colorless area after a time spreads, and at the same time the group of Paramecia begins to break up, as previously described. The Paramecia swim back and forth in the colorless area (that is, the area containing (CO_2), from one side to the other, but without passing its boundaries. The colorless area increases in size, and the area in which the Paramecia swim back and forth keeps exact pace with it; the two coincide throughout.

The same phenomena may be produced by introducing a small bubble of CO_2 into the slide. The Paramecia collect closely about the CO_2, pressing against the bubble. In this way a dense mass is soon formed. After a time, as the CO_2 diffuses, the mass loosens; the Paramecia swim back and forth in the area of diffusing CO_2, not overpassing its boundaries. The phenomena caused by the presence of a bubble of CO_2 are identical in every respect with those which are apparently spontaneous. There is no question but that the assembling of the Paramecia into crowds is due to the presence in these crowds of CO_2 excreted by the animals themselves.

Thus it appears that our social phenomena, with all their implications of higher mental powers, have evaporated into a simple attraction toward carbon dioxide.

But how do the animals succeed in collecting from a distance? At first they are distributed throughout the entire preparation; when we introduce the bit of bacterial zoogloea or filter paper, how do the Paramecia discover its presence, so as to collect about it? From the general wreck of higher mental qualities, can we not save at least the *acute senses* necessary to account for these phenomena?

To determine how the Paramecia succeed in finding and collecting about a small solid placed in the middle of a large slide, it is necessary to study the ordinary method of locomotion of the animals. "If a preparation of Paramecia on a slide, containing in one spot a small bit of filter paper is closely observed, the Paramecia are seen at first to swim hither and thither in every direction, apparently without directive tendency of any sort. . . Soon a single individual strikes in its headlong course the bit of paper. It stops at once, often starts backward a slight distance, and whirls about on its short axis two or three times, then settles against the bit of paper and remains. Quickly another and another strike in the same way and remain. Now the excretion of CO_2 by the animals gathered together begins to take effect; the region becomes a strong center of attraction, and in ten to fifteen minutes, and often less, the paper is surrounded by a dense swarm of Paramecia, containing a large majority of all those in the preparation." (I, p. 299.) Thus, the finding of the bit of paper is due simply to the roving movements of the animals. Moreover, for gathering in an area containing CO_2 or other acid alone, a similar dependence upon chance motions appears. There is no swimming in straight radial lines to the area of CO_2 as a center; the Paramecia swim at random until they come by accident into the region of CO_2; there they remain. The precise place where a group of Paramecia is formed in some part of a slide into which nothing has been intentionally introduced that would act as a center of attraction is determined by chance factors. One or two individuals, perhaps, strike by accident a bit of solid matter suspended in the water, or a slight roughening of the cover glass; the thigmotactic reaction is set up, so that they stop, and as a result the region becomes a center for the production of carbon dioxide. The remainder of the collection is then due to CO_2, and takes place in the manner last described.

We must, therefore, along with the rest, dispense with specially acute senses. The Paramecia do not react until they are in actual contact with the source of stimulus, and for coming in contact with the source they depend upon roving movements in all directions.

Thus we find that all more complex psychological powers deduced from the "social phenomena," as well as those from the choice of food, must fall to the ground. For explaining all the phenomena with which we have thus far dealt, but three factors are necessary: (1) the customary movements of the unstimulated animal; (2) the cessation of these movements, except those in the oral groove and gullet, when in contact with solids of a certain physical character; (3) attraction toward CO_2.

We have still remaining to be accounted for psychologically the *attraction* toward certain reagents and conditions, as toward CO_2 and toward the optimum temperature, and the *repulsion* toward other reagents and conditions, such as alkalies, and cold or great heat. This selective attraction and repulsion is a phenomenon of great importance, seeming in itself to imply a *choice* on the part of the organisms. If they move toward certain sources of stimuli and away from others, this seems to involve a perception of the localization of things, and this can hardly be regarded otherwise than as at least the beginnings of intelligence. Moreover, from its apparent general occurrence, much theoretical significance has been attached to it. Now *how* does this attraction and repulsion take place? Organisms usually move by means of certain organs of locomotion; attraction and repulsion cannot therefore be left as abstract ideas, but it must be shown how the attractive agent sets these organs in operation in such a manner as to bring the animal nearer; how the repellent agent succeeds in affecting the locomotor organs so as to carry the animal away. To apply this to the particular case in hand, when a drop of some attractive solution is introduced into a slide of Paramecia, how does it succeed in affecting the cilia of the animals in such a way that they turn toward and enter the drop?

Exact observation of the method by which the Paramecia enter such a drop shows that this question is based on a false assumption. *The animals do not turn toward the drop.* Such a drop diffuses slowly, so that its margin is evident, and the Paramecia may be seen in their random course to almost graze the edge of the drop without their motion being changed in the slightest degree; they keep on straight past the drop and swim to another part of the slide. But of course some of the Paramecia in their random swimming come directly against the edge of the drop. These do not react, but keep on undisturbed across it. But when they come to the opposite margin, where they would, if unchecked, pass out again into the surrounding medium, they react *negatively*—jerking back and turning again into the drop. Such an animal then swims across the drop in the new direction till it again comes to the margin, when it reacts negatively, as before. This continues, so that the animal appears to be caught in the drop as in a trap. Other Paramecia enter the drop in the same way and are imprisoned like the first, so that in time the drop swarms with the animals. As a result of their swift random movements when first brought upon the slide, almost every individual in the preparation will in a short time have come by chance against the edge of the drop, will have entered and remained, so that soon all the Paramecia in the preparation are in the drop. If, however, the drop is not introduced until the Paramecia have quieted down, it will be found to remain empty; this shows the essential part played by the roving movements in bringing the collection together.

Thus it appears that the animals are not attracted by the fluid in the drop; they enter it by chance, without reaction, then are repelled by the surrounding fluid. This is true for all apparently attractive reagents or conditions. *Paramecia are not directly attracted by any substance or agency;* the assembling in the region of certain conditions being due to the repellent power of the surrounding fluid, after the Paramecia have entered by chance the area of the conditions in question.

There remains then as a motor reaction only the *repulsion* due to certain agents and conditions. Is this repulsion an ultimate fact in the psychology of the animal, or is it possible to analyze it further?

The first thing which a Paramecium does on coming in contact with a drop of repellent solution is to reverse all its cilia, so as to swim straight backward,—at the same time revolving on its long axis in a direction opposite to that in which it was previously revolving. Next it turns to one side a certain amount, then swims forward again, on a path which lies at an angle to the path in which it was first swimming. Briefly stated, it adopts the very rational plan of backing off, turning to one side, and swimming on past the obstacle. We must apparently concede the Paramecium at least a modicum of intelligence for the very practical way in which it meets this emergency.

But suppose the animal touches the margin of the drop obliquely, or brushes it only on one side as it swims past it through the water; what course will it then take? From its sensible behavior under the previous conditions we shall expect it to sheer off, away from the drop, and keep on its way undisturbed or at a slight angle to the original path. But when we observe such a case, we find that the Paramecium backs off, swimming straight backward, as before, then turns through an angle, then swims forward, exactly as in the previous case. And curiously enough, it by no means turns directly away from the drop, but fully as often turns toward it, so as to strike it squarely the next time it moves forward. If this occurs, the whole operation is repeated; the animal tries, as it were, for a new opening. Sometimes it is necessary to repeat the operation several times before the Paramecium succeeds in getting away from the repellent object.

Under these circumstances the animal evidently gives much less indication of intelligence, and the fact that it reacts in exactly the same way under such different conditions is especially fitted to shake confidence in its mental powers. Apparently the swimming backward has no relation to the position of the source of stimulus, but occurs merely as a result of the fact of stimulation, without reference to its localization. Whether this is true as a general statement can be tested by giving the animal a general shock without localizing the source of stimulus at all. This is easily done by immersing the Paramecia directly into solutions of such a nature that they act as stimuli. In such a case the stimulus acts upon the entire surface of the animal at once, so that there is no obstacle to be avoided and no reason for swimming backward.

Immersing Paramecia thus into solutions of different kinds, it is found that the first thing they do in every case is to reverse the cilia and swim backward. Nor is this all. The entire reaction is given, just as when the source of stimulus was at one end or one side; the animal first swims backward, then turns, then swims forward. This is true for all classes of stimuli,—chemical solutions of all sorts, water heated considerably above the optimum temperature, water at the freezing point, and solutions active only through their osmotic pressure. The duration of the different parts of the reaction varies much in different agents, but the essential features of the reaction are the same everywhere.

It therefore appears that not only the backward swimming, but also the turning to one side takes place without reference to the localization of the stimulus,—both occurring equally when the stimulus is not localized at all. But what determines the *direction* in which the Paramecium turns?

Careful observation of Paramecia under conditions which compel them to move slowly shows that after stimulation *they always turn toward the aboral side*,—that is, the side opposite the oral groove. The direction of turning is thus determined by the structure of the animal, and has no relation to the position of the source of stimulus. The mechanism of the turning is as follows: after the first reversal of cilia, those in the oral groove begin to strike backward again, tending to drive the animal forward, while the remainder of the cilia on the anterior half of the animal *strike transversely toward the oral side*. This results in turning the animal toward the aboral side.

We find, therefore, that the direction of motion throughout the entire reaction depends upon the structure of the animal and has no relation to the localization of the stimulus. The reaction may be expressed completely, omitting all reference to the position of the stimulus, as follows: after stimulation the animal swims with the more pointed end in front, turns toward the aboral side, then swims with the blunter end in front.

It is of course a matter of chance whether this turning toward the aboral side carries the animal away from the source of stimulus or toward it. Frequently the latter is true; in this case the operation is repeated when the animal comes again in contact with the stimulating agent. As the animal revolves continually on its long axis, the aboral side will probably lie in a new position at the next turning, so that the animal will turn in a new direction. If this is repeated, the chances are that in time the obstacle will be avoided.

Thus, not only is it true that Paramecium is not attracted by any agent or condition, but also we cannot say, speaking strictly, that it is repelled by any agent or condition. Certain agents set up a reaction in the animal, the directive features of which depend entirely upon the structure of the organism,—just as certain stimuli cause an isolated muscle to react. We cannot say that the Paramecium is repelled by the stimulus, any more than we can say that the contraction of the muscle is due to the muscle's being repelled by the stimulus. It is true that the source of stimulus is more often at the blunt or "anterior" end, in the case of Paramecium, so that swimming toward the sharp end does, as a matter of fact, usually result in taking the Paramecium away for a short distance from the source of stimulus. But this usual position of the source of stimulus is from a physiological standpoint purely accidental, and the reaction produced is the same whether it occupies this position or another. If the animal is stimulated at the posterior end, it swims backward, therefore toward the source of stimulus; in this way it may enter a destructive chemical solution and be immediately killed, though the same chemical acting upon the anterior end would of course have caused the animal to swim away. This is seen in a particularly striking manner in the larger infusorian *Spirostomum ambiguum*, which is so large that it is easy to apply a stimulus to any desired part of the body. It is then found that the animal reacts in exactly the same manner whether stimulated at the anterior end, the posterior end, or the side, the direction of motion having absolutely no relation to the position of the source of stimulus. The same is true for Paramecium, though its smaller size makes the demonstration more difficult.

A strict parity is therefore to be observed between the reactions of Paramecium and those of an isolated frog's muscle. Paramecium responds to any stimulus by a definite, well characterized reaction: "The same may be said of the isolated muscle of a frog. The intensity of the reaction varies with the nature and intensity of the stimulus; this also is true for the muscle. Under certain influences the Paramecium remains quiet; likewise the muscle. The directive relations of the motions are determined both in the Paramecium and in the muscle by the structure of the organism, not by the position of the source of stimulus. There seems, then, no necessity for assuming more in order to explain the reactions of the Paramecium than to explain the reactions of the muscle. We need, therefore, to assume nothing more than irritability, or the power of responding to a stimulus by a definite movement, to explain the activities of Paramecium" (II). The long catalogue of psychical qualities required to account for the movements of Paramecium is thus reduced to simple protoplasmic irritability.

The method by which Paramecia collect in the regions of influences of a certain character and leave other regions empty, may be stated in general terms as follows: Certain stimuli cause in the animals random motions, in which the direction is frequently changed, especially at the moment when the stimulus begins to act. These random movements result, through the laws of chance, if continued long enough, in carrying the Paramecia out of the region of influence of the agent causing the stimulus. Coming thus by chance into a region where such movements are not caused, the Paramecia remain; if this ineffective area is small, the Paramecia are crowded together within it and give the impression of being strongly attracted by it.

"It is evident that we have in this case as near the reaction postulated by Spencer and Bain for a primitive organism—namely, random movements in response to any stimulus—as is likely to be found in any organism. The motions are strictly random in character so far as the position of the source of stimulus is concerned. . . . And by the repetition of the reaction the direction of movement is frequently changed,—always without reference to the localization of the stimulus. It appears not to have been foreseen theoretically that such random movements would of themselves, if continued, carry the animal out of the sphere of influence of the agent causing them and keep it from re-entering. To accomplish this result it is only necessary that the direction of motion should be changed at the moment when the stimulus begins to act and at intervals so long as it continues" (II).

An examination of the activities of a number of other unicellular organisms in the light of the observations above detailed shows that they react in essentially the same manner. For each organism a simple statement can be given for the reaction to any stimulus. For *Spirostomum ambiguum* the reaction is as follows: the animal contracts, swims backward, turns toward the aboral side, and swims forward. *Stentor polymorphus* contracts, swims backward, turns toward the *right* side, and swims forward. A number of flagellates also have been found to have such a fixed method of reaction. In all these cases the direction of motion has no relation to the position of the source of stimulus, and the conclusions to be drawn for Paramecium apply equally to these organisms.

In regard to the position in the psychological scale to be assigned to Paramecium the following may be said: The reactions of Paramecium are, as we have seen, comparable in all essentials to those of an isolated muscle. In neither case has the direction of motion any relation to the position of the source of stimulus. Reaction in such a manner as to show a relation to the position of the stimulating agent has rightly been regarded as a first and lowest step in perception; this lowest step is quite lacking in Paramecium. Moreover, Paramecium has no "life history" in the sense of a change in its reactions such as between the reactions of a young and an adult higher animal. An individual undergoing division reacts exactly like the ordinary Paramecium, as do likewise the halves immediately after division. In the words of Professor Baldwin, "the fact of life history is just what distinguishes an organism from what is a 'mechanical arrangement.'" While we cannot deny that Paramecium is an organism, this fact shows the machine-like nature of its activities. An animal that learns nothing, that exercises no choice in any respect, that is attracted by nothing and repelled by nothing, that reacts entirely without reference to the position of external objects, that has but one reaction for the most varied stimuli, can hardly be said to have made the first step in the evolution of mind, and we are not compelled to assume consciousness or intelligence in any form to explain its activities.

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REACTIONS OF INFUSORIA TO CHEMICALS: A CRITICISM.

H. S. JENNINGS.

In the January number of the *American Journal of Physiology*, Garrey[1] gives the results of extended experiments on the effects of chemicals in causing motor reactions in the flagellate Chilomonas. From the chemical side the paper is a valuable one, as being the most thorough study yet made of the specific effects of chemicals in causing reactions in unicellular organisms, and in treating the subject from the standpoint of modern physical chemistry. On the other hand, that portion of the paper which treats of the part played in the reactions by the organisms seems to me much less successful, and it is my purpose to point out certain criticisms on this part of the work.

Garrey found that the motor reactions of Chilomonas are set strongly in operation by certain chemicals, "very swift shooting movements being induced," so that the organisms, as a result of these movements, soon leave the sphere of influence of the substance in question. This effect is termed by Garrey "chemokinesis"; it is analogous to that which I have called "negative chemotaxis" in Paramecium. The chief portion of the paper is devoted to determining the relative strength of various chemicals in causing this reaction, and the exact factors in the solutions that produce the effect; interesting and valuable results are here brought out.

In certain organic acids (acetic, butyric, lactic) and their salts Garrey found the flagellates to form aggregations, as Paramecium does in solutions of CO_2 and acids of all sorts;[2]

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[1] Garrey, Walter E. The Effects of Ions upon the Aggregation of Flagellated Infusoria. *Amer. Journ. of Phys.*, vol. iii, pp. 291–315.

[2] Owing to the failure of his attempts to repeat my experiments with solutions of CO_2 and inorganic acids, Garrey denies my results with Paramecia. His failure was unquestionably due to neglect to fulfill the necessary experimental conditions. I have never known the experiments to fail when properly carried out.

these aggregations, he says, are due to "true chemotropism." In that portion of the work which deals with the way in which these aggregations are formed and with the part played by the organism, it seems to me that Garrey neglects certain facts that are absolutely fatal to the view of these matters which he puts forth. He did not determine the mechanism of the reactions of Chilomonas, — the exact way in which the organism moves when stimulated, — and this seems to me the first requisite for an understanding of how the reactions are brought about. Garrey's point of departure is his definition of *tropism* (which he says is the same as *taxis*).

Taking heliotropism as a type, he says: "In heliotropism the organism is so oriented that its axis or plane of symmetry coincides with the direction of the rays, and symmetrical points on the surface of the body are struck by the light rays at the same angle." He then quotes with approval, for the case of chemotropism, Professor Loeb's generalization: "The essence of chemotropic orientation would then consist in the animals placing themselves in such a position that symmetrical points on the surface of the body are cut by the diffusion lines at the same angle." The movement of Chilomonas into drops of organic acid, Garrey says, is "true chemotropism," and he states that the organisms move toward the center of diffusion "as if orienting themselves to radially disposed lines; lines which may represent the paths of diffusing molecules or ions." From this one might infer that Chilomonas actually does so orient itself "that symmetrical points on the surface of the body are cut by the diffusion lines at the same angle," and then swims forward in this position, as required by the generalization; Garrey says nothing to the contrary. Yet the animal does nothing of the sort. The very elementary fact seems to have escaped Garrey that Chilomonas, as is well known, is *unsymmetrical*, so that it is impossible for it to orient itself in this manner; there *are* no such symmetrical points to be thus cut. (In Chilomonas the so-called "upper" or larger lip lies at the dorso-dextral angle of the anterior end, as will be seen by consulting Fig. 9 *b* of Pl. XLV of Bütschli's *Protozoa*, so that the animal is unsymmetrical both dorso-ventrally and dextro-sinistrally.) Furthermore, observation of its movements shows that it makes no attempt to do the impossible; instead of retaining any such position as the generalization calls for, it swims in spirals, a certain side of the animal being always toward the inside of the spiral; as Bütschli says, they "describe rather narrow circles" (Bütschli, *Flagellata,* p. 853). These facts, of the lack of symmetry of the organism, and its swimming in spirals, might be thought to be facts of capital importance for a decision as to whether it falls under Professor Loeb's generalization or not; they are nowhere mentioned by Garrey. It is well known that the Infusoria are prevailingly unsymmetrical, so that it is quite impossible for most of them to fall within this generalization. An attempt to apply it to one of the Hypotricha, for example (see figure on p. 262), where not only the form of the body but the structure and distribution of the locomotor organs are strikingly unsymmetrical, would bring out the absurdity of the attempt, and observation of the actual movements of the organisms, as detailed in the Fifth of my Studies,[1] only accentuates the impossibility. It may be regarded as axiomatic that a principle which requires the symmetry of organisms cannot be applied to organisms that are unsymmetrical. On this account I have not previously considered it worth while, in my Studies on Reactions, etc., to show the relation of Professor Loeb's well-known generalization to the movements of the prevailingly unsymmetrical Infusoria. The generalization was evidently made from a study of bilaterally symmetrical animals, and may be of the greatest value for an interpretation of their activities; it is obviously inapplicable to unsymmetrical organisms.

I have described the mechanism of the motor reactions of Chilomonas, on pp. 231–234 of the same number of the *American Journal of Physiology,* as that in which Garrey's paper appeared. Later study of their reactions in the production of the aggregations described by Garrey has convinced me that these aggregations are produced through the mechanism of the described motor reaction, in a manner exactly analogous to the production of the similar aggregations of Paramecium in weak acids, described on pp. 314, 315, of the Second of my Studies.[1] This possibility is not discussed by Garrey, apparently because of a preconceived view that chemotropism must take place according to Professor Loeb's generalization above quoted. He says: "Jennings's motor reaction cannot account for orientation," and, therefore, that it has nothing to do with tropisms. This unsupported sweeping denial seems a peculiar way of meeting such a detailed account of the production of orientation through the motor reaction as I have given for the thermotaxis of Paramecium on pp. 334–336 of the Second of my Studies. The general proposition that a motor reaction cannot cause orientation seems still more remarkable. Consider an organism, as in the figure, lying obliquely to the line of action of the incoming stimulus, which is indicated by the four straight arrows. Its position is at first *a–a*. After the stimulus has acted for a time, the organism is found to be oriented as shown at *b–b*, with its longitudinal axis in the direction of the lines of force. Now, how can this orientation possibly take place *except* through a motor reaction? The animal has certainly moved under the influence of the stimulus from the position *a–a* to the position *b–b*, and such a movement under the influence of a stimulus is what is commonly known as a motor reaction.[2]

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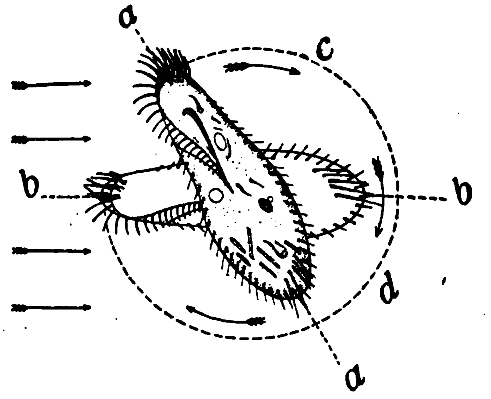
[1] *Amer. Journ. of Phys.,* vol. iii, p. 249.



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[1] *Amer. Journ. of Phys.*, vol. ii, pp. 311–341.

[2] Garrey's attempt to attribute to me the absurd idea that the principle of a motor reaction is new (*loc. cit.*, p. 313, note), is perhaps unworthy of mention. The comparison of the motor reaction of Paramecium with that of a muscle,

The essential feature of the characteristic motor reaction of these organisms, as I have described it, is that they turn, when they do turn, toward a structurally defined side, whatever the line of action of the stimulus. For example, the organism in the figure (one of the Hypotricha) turns to the right, in the direction indicated by the curve *c–d*. But evidently the organism may come into the position *b–b* as easily by turning to the right as by turning to the left; in the former case it must simply keep turning somewhat longer. As a matter of fact, orientation to most stimuli occurs in the Hypotricha in exactly the manner illustrated — by turning to the right. This is true for chemical, osmotic, mechanical, and thermal stimuli; whether for electric stimuli and light has not been shown.

The precision of the orientation, and whether a large number of organisms will be oriented in the same way, depends upon the question as to what influences produce the motor reaction. Suppose that in the figure the stimulating agent is something that acts steadily in lines coming in the direction of the straight arrows. Now, I have shown in the Fifth of my Studies that many of these organisms are much more sensitive at the anterior end than elsewhere, so that a stimulus at the anterior end produces a different effect from a stimulus elsewhere on the body. When, therefore, the organism is so oriented that the lines of force impinge directly on the anterior end (position *b–b*), it will, of course, be differently affected as compared with a position in which the sensitive anterior end is wholly or partly protected by the remainder of the body. Thus different reactions may be caused in the two cases; in one position the motor reflex may be caused, in the other not. If, for example, the motor reflex is caused when the lines of force impinge on even a small part of the anterior end, then the organism will not cease giving the reaction until it is oriented with anterior end directed away from the side of incoming

which Garrey makes for my information, I had already developed at length to show their identity in character, in two papers (*Amer. Journ. of Phys.*, vol. ii, p. 339; *Amer. Journ. of Psychol.*, vol. x, p. 513). I had counted it a chief result of my work that it had reduced the supposed attractions and repulsions of these organisms to a motor reflex "of the same order as the motor reflexes of higher animals," as I have expressed it elsewhere.

force. If a large number of such organisms are in the field of the force, they will soon all show a common orientation, and when they move they will all move in the same direction. *All that is necessary for orientation to take place in this way is that the organism shall be differently affected when it is in one position from the way it is affected in another position.* It is difficult to imagine any agency that can cause orientation in which this condition is not fulfilled.

When the organism is once oriented with reference to a constant stimulus, of course no further reaction is necessary; it simply keeps up the normal forward motion until this motion brings it into new relations with the stimulus, when the motor reaction is again caused, resulting in a readjustment of orientation. In the hypothetical case above supposed, if the organism swims in a curve instead of a straight line, it will soon come into a position such that the lines of incoming force impinge on the sensitive anterior end; the motor reaction is thereby induced until the animal has come again into the position where the anterior end is not thus affected.

Garrey says: "All these motor reactions have nothing to do with *tropisms*, for these motor reactions are only the expression of a very sudden change in the stimuli, while the characteristic of the tropism is the stationary condition of the stimuli." He fails to consider the fact that, stationary as the stimuli may be, the organisms with which he is dealing are *not* stationary; by their own movements the organisms come into new relations with the stimuli, and these new relations may result in the production of the motor reaction, causing a readjustment of orientation.

To summarize, the chief points which I would make in criticism of Garrey's conclusions are as follows:

1. In all work upon the reactions of organisms to physical and chemical agents, it is necessary to consider the structure and normal activities of the organisms, as well as the nature of the agents acting as stimuli.

2. Orientation such that "symmetrical points on the surface of the body are cut by diffusion lines at the same angle" (or by any other lines at the same angle) is impossible in unsym-

metrical organisms, such as are a large proportion of the Infusoria.

3. Observation of the movements of the Infusoria, both the unsymmetrical and the symmetrical, shows that they do not orient themselves even approximately in the manner above expressed, but that they swim as a rule in spirals, and orientation usually takes place through a motor reflex, characterized by the fact that the organism, when it turns, turns toward a structurally defined side.

4. A sudden change in the stimulus is not necessary to produce a motor reaction, as Garrey has stated, for the organism itself, by moving, comes into new relations with a constant stimulus, and by these new relations the motor reflex may be caused, resulting in a readjustment of orientation.

5. The gatherings of Chilomonas in drops of organic acid, described by Garrey, take place, according to my observations,[1] through the mechanism of the motor reaction of Chilomonas, described in the Fifth of my Studies. Whether this is "true chemotropism" or not depends on the definition of chemotropism.

6. The orientation of Chilomonas takes place through this motor reaction, and in general it is impossible for an unoriented organism to become oriented except through a motor reaction of some sort (except where passively moved, like a dead body).

ANN ARBOR, MICH., Feb. 6, 1900.

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[1] The observations on which this statement is based are detailed in a paper by the author in the April number of the *American Journal of Physiology*.

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U. S. COMMISSION OF FISH AND FISHERIE
GEORGE M. BOWERS, Commissioner.

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A REPORT OF WORK ON THE PROTOZOA O

WITH ESPECIAL REFERENCE TO THE LAWS OF THEIR M

BY

H. S. JENNINGS,
Instructor in Zoology in the University of Michigan

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1900.

CONTRIBUTIONS TO THE BIOLOGY OF THE GREAT LAKES.

A REPORT OF WORK ON THE PROTOZOA OF LAKE ERIE, WITH ESPECIAL REFERENCE TO THE LAWS OF THEIR MOVEMENTS.*

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The general plan of work outlined in the preceding paper for a study of the Rotatoria in their relations to the life conditions of the lake would apply equally, with some changes, to the Protozoa or to almost any other group of animals. In the study of the Protozoa it was decided to strike at once for the heart of the matter by beginning an investigation of the laws of their activities. A typical and very abundant Infusorian, *Paramecium caudatum*, was selected for special examination, and a study was made of the nature of its activities and the laws which govern them, in the hope that a full knowledge of such laws in the case of one Protozoan might furnish a key to an understanding of the activities of other members of the group, as well as, in time, to those of higher animals. The results of this work have already been published in detail elsewhere, so that a brief résumé is all that will be presented here.

The work was successful in determining the general mechanism of the reactions of Paramecium to changes in the external conditions, and in showing that the reactions are of the same essential nature in many other Protozoa. It was shown that the reactions of Paramecium are of extraordinary simplicity. As will be recalled by most biologists, Paramecium is a somewhat cigar-shaped animal, with one end narrow and blunt, the other broad but pointed. From the blunt end a groove passes obliquely along one side of the body to the middle, ending there in the mouth. The side on which the mouth and groove lie may be distinguished as the oral side; the opposite side (on which the contractile vacuoles lie) as the aboral side. The entire surface of the animal is covered with cilia, by means of which it moves. As it more usually moves in the direction of the narrower, blunter end, that may be called the anterior end, the other the posterior end.

In what might be called the normal condition of affairs all of the cilia strike backward, so that the animal moves forward; at the same time it revolves on its long axis. Now, when a change is produced in the environment of the Paramecium, such as by it striking against an obstruction or passing into water of different chemical content or different temperature, the normal activity is modified in one of two ways:

1. If the Paramecium comes in contact with a solid body of a loose fibrous texture, its activity is decreased; the cilia on the surface of the body cease their

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* The papers in this series are based on investigations of the U. S. Fish Commission under direction of Prof. Jacob Reighard, of the University of Michigan. The following have already appeared:

The Mechanism of the Motor Reactions of Paramecium. <Am. Jour. Physiology, vol. II, pp. 311-341.

Laws of Chemotaxis in Paramecium. <Am. Jour. Physiology, vol. II, pp. 355-379.

movement, while those in the oral groove and the gullet continue active. As a consequence locomotion ceases; the animal comes to rest against the solid body, while the cilia of the oral groove continue to drive a stream of water to the mouth. This reaction to a solid body may be called thigmotaxis. If the loose solid body is a mass of bacterial zoogloea, the stream of water carries numbers of bacteria to the mouth, where they pass into the internal protoplasm of the animal and are digested; thus Paramecium gets its food. But the animal conducts itself in exactly the same way toward other loose fibrous bodies, such as bits of cloth, paper, sponge, or the like, the presence or absence of material that will serve as food having nothing to do with the production of the reaction. On the other hand, the substances held in solution in the water have a marked effect on the tendency of the Paramecia to react in the manner above described. If the water is faintly acid in reaction, the Paramecia are much more inclined to come to rest as just described. This is especially noticeable in water containing carbon dioxide. The presence in the water of an alkali in solution has, on the other hand, precisely the opposite effect, tending to inhibit the thigmotactic reaction.

2. Any other change in the conditions, of such a nature as to act as a stimulus, causes a definite change in the movements, which is of a stereotyped character, being of the same nature for almost every stimulus. When stimulated, Paramecium swims backward, turns toward its own aboral side, then swims forward again. The same reaction is produced by stimuli of the most varied kinds—by acids, alkalis, and neutral salts, by heat, by cold, by mechanical shock. The reaction is the same whether the stimulus first affects the anterior end, the posterior end, one side, or the entire surface of the animal at once. The direction in which the animal swims has thus no relation to the localization of the stimulus. If the stimulus comes from the anterior end, swimming backward of course carries the animal away from it; if the same stimulus comes from the posterior end, swimming backward carries the animal toward it. If an injurious chemical substance diffuses in such a way as to first come in contact with the posterior end of a resting Paramecium, the latter therefore swims backward directly into the substance and is killed. The turning is likewise without relation to the position of the stimulus. The animal always turns toward its own aboral side, so that the absolute direction in which it turns depends upon the chance position of the aboral side when the turning begins. As the animal continually revolves, both when swimming forward and when swimming backward, it is impossible to predict in which direction the aboral side will lie after the animal has swum backward a distance from its position when stimulation occurs; and observation shows that when Paramecium strikes on one side against an obstruction, it is fully as likely to turn toward the obstruction, after swimming backward, as to turn away. In the former case it of course strikes the obstruction again; the whole reaction is then repeated. Owing to the continual rotation on the long axis, the aboral side will probably be in a new position next time, so that the animal will turn in a new direction. If this repetition is continued, the Paramecium is certain finally, by the laws of chance, to avoid the obstacle.

The factors determining the direction of motion in Paramecium are thus internal; the direction of its movements has no relation to the position of external objects. This result is of fundamental significance for interpretation of the movements of these creatures, and throws a flood of light on many of the phenomena of their life. Study of some other Infusoria in the light of the result on Paramecium has shown that the same is true for these. *Spirostomum ambiguum* when stimulated contracts, swims backward, turns toward the aboral side, and swims forward. If touched with a spicule of glass at its anterior end it swims backward, *away* from the glass; if touched at the posterior end it likewise swims backward, therefore *toward* the glass. *Stentor polymorphus* when stimulated contracts, swims backward, turns to the right, then swims forward, the direction of motion having, as in the other cases, no relation to localization of the stimulus. Similar results, not yet published in detail, have been obtained with certain Flagellata, as well as with other Ciliata.*

Besides determining the general mechanism of the reactions of Paramecium, an extended study was made of the effects upon its activities of different chemicals. If into a preparation of Paramecia mounted upon the slide a drop of some chemical substance in solution is introduced, the Paramecia may either collect in the drop or leave it entirely empty. In the former case they show positive chemotaxis to the substance; in the latter case, negative chemotaxis. As to what might be called the mechanism of chemotaxis, the following was made out. The Paramecia are not attracted by the drop of substance into which they gather. They may graze the very edge of the drop without swerving a particle from their course so as to pass into it. But Paramecia when first brought upon the slide swim rapidly in every direction, so that many will quickly come by chance against the edge of such a drop. They do not react, but swim straight ahead—the substance in the drop not acting as a stimulus to produce the motor reaction above described. But on passing across the drop, the outer fluid does, after the Paramecia have been in the drop of the chemical, act as a stimulus to produce the motor reaction. The Paramecium therefore, on coming to the outer edge of the drop, swims backward, thus returning into the drop. It then turns toward the aboral side and swims forward (in accordance with the general scheme of reaction above described). It thus comes to the outer edge of the drop once more; reacts again, and this being kept up, is continually prevented from crossing the boundary of the drop into the surrounding water. The same process is repeated for many Paramecia, until in time the drop swarms with the Infusoria, so that they appear to have been attracted into it.

In case of a substance in which the Paramecia do not collect at all, observation shows that the motor reaction (swimming backward, turning, and swimming forward) is produced when the Paramecia come against the drop from the outside; hence they are prevented from entering and the drop remains empty.

It was found possible to classify chemical compounds thus into two classes. On the one hand may be placed together those which do not produce the reaction when the Paramecia enter them, but throw the animals into such a physiological condition that they do react when they pass out of a drop containing the substance in question. In these the Paramecia, therefore, gather and are said to be positively chemotactic toward them. On the other hand are substances which produce the motor reaction as soon as the Paramecia come in contact with them, so that the animals do not enter solutions of these compounds at all, and may be said to be negatively chemotactic toward them.

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* The reactions of a large number of Protozoa have been studied since the above was written. In all of these the direction of turning was found to be determined entirely by internal factors, and to have no relation to the position of the source of stimulus. The direction of motion along the body axis, on the other hand, was found in a number of cases, for mechanical stimuli, to be determined by the localization of the stimulus. *Loxodes rostrum*, for example, when touched with a glass rod at the anterior end swims backward; touched at the posterior end it swims forward. For chemical stimuli, however, the *absence* of any such dependence of the direction of movement on the localization of the stimulus was demonstrated. For details, see a paper by the author on "The movements and motor reflexes of the Flagellata and Ciliata," in the American Journal of Physiology, January, 1900.

In the former group (substances toward which the Paramecia show positive chemotaxis) belong all acids, and salts whose solutions have an acid reaction or contain hydrogen ions, as in salts of the heavy metals. In the group of substances toward which the Paramecia are negatively chemotactic belong all alkalies and substances having an alkaline reaction, as well as almost all compounds which contain ions of the alkali and earth alkali metals in their solutions. Certain substances take an intermediate place. Containing the ions of an alkali or earth alkali metal, they produce the motor reaction when the Paramecia enter a drop of fluid containing them; but having likewise hydrogen ions, they also cause the animals to react when they leave the drop. Examples of such substances are potassium and ammonium bichromate. In these cases the hydrogen ions seem to be active (in their characteristic way) in a more dilute solution, and, therefore, farther from the center of a diffusing drop than are the ions of the metals. The Paramecia, therefore, enter the outer margin of the drop and are unable to leave it, while at the same time they are unable to pass to the center of the drop. They thus gather in a ring about the drop, leaving the center empty.

The classification of substances into those toward which the Paramecia are positively chemotactic on the one hand and those toward which they are negatively chemotactic on the other, thus follows the lines of a chemical classification; the former including acids, the latter alkalies and salts of the alkali and earth alkali metals.

Experimentation showed that the relative injuriousness of solutions has comparatively little to do with the nature of the chemotaxis. Paramecia are repelled strongly by many substances that are scarcely injurious at all, while they enter without hesitation other substances in which they are at once killed. The repellent powers of different chemical compounds are in no way proportional to their injurious effects.

The researches on chemotaxis have thus far been restricted almost entirely to Paramecium, but the general laws obtained for this animal promise to throw much light on related phenomena in others.

As described above, positive and negative chemotaxis, or the collecting in or avoidance of certain chemicals, takes place through the mechanism of the general motor reaction first described. The only activity of the Paramecia concerned in it all is the swimming backward, turning toward the aboral side, then swimming forward, when stimulated. The qualitative differences that seem apparent in their reactions toward different substances depend merely upon what does and what does not act as a stimulus.

The mechanism of collecting in or avoiding agencies or conditions, other than chemical, is exactly the same as that just described. In the case of temperature, for example, certain grades of heat or cold produce motor reaction, so that the Paramecia do not enter these; or, if already within a zone of such temperature, they continue moving about violently till a chance movement carries them into a region where the temperature is not such as to cause a reaction; there they remain. In general, therefore, the Paramecia gather and remain in substances or conditions which do not cause the motor reaction, while they leave empty such substances or conditions as do cause their one motor reaction. It follows that they collect in regions of a certain temperature, avoiding great heat or cold, and that they collect in water holding in solution substances of an acid character, avoiding alkaline solutions. Under natural conditions, their collecting into regions acidulated by carbon dioxide excreted by themselves is particularly noticeable. It results in bringing the Paramecia together in dense swarms. To this is to be added, as a second factor in bringing the Paramecia together, the fact that contact with solids of a loose fibrous texture likewise tends to quiet the Paramecia, so that they collect about such solids. In the fluids in which the Paramecia live, such solids are present as masses of bacterial zoogl&oelig;a, upon which the Paramecia will usually be seen to be collected in swarms.

The ordinary life of a Paramecium may then be summarized somewhat as follows: In the free water, as long as the animal is unstimulated, it swims forward in a spiral course, revolving on its long axis. But it comes in contact here and there with changes in the environment—regions of higher or lower temperature, or of greater or less amounts of certain chemicals in solution, or with mechanical obstructions. If these changes are of such a nature as to act as a stimulus, the Paramecium thereupon swims backward a short distance, turns toward one side (in a direction which is an entirely random one so far as outer objects are concerned) and continues forward. This reaction is repeated as often as the Paramecium comes in contact with any source of stimulus. Certain solutions or conditions cause no reaction. Thus the Paramecium may pass by chance into a group of other Paramecia, where the water is charged with carbon dioxide, which they have excreted. Now, the surrounding water containing *no* carbon dioxide causes the reaction, so the Paramecium remains with the others. Or, if it comes in contact with a loose, soft body it stops, only the oral cilia continuing to be active. These constantly carry a stream of water to the mouth, and if the solid is by chance a bit of bacterial zoogl&oelig;a, this stream carries many bacteria into the mouth of the animal, so that they serve as food. But if no bacteria are present, the Paramecium nevertheless remains indefinitely against the bit of solid, especially if joined by other individuals, so that they are in a region containing carbon dioxide (excreted by themselves).

This resting condition may be brought to an end by too high or too low a temperature, by the diffusion of an alkali into the water, or by various other conditions that tend to produce a motor reaction. The Paramecium then continues on its way in some chance direction till it comes again into conditions which do not act as a stimulus.

Thus the life of the animals is extremely simple; they have but one mode of reaction to outer conditions—by swimming backward, turning, swimming forward—while under other conditions their activity largely ceases.

It is obvious that such simple activities, while they do result in keeping Paramecia out of certain conditions and bringing them into others, would not be adequate for preserving the animals under complicated and changing conditions. The Paramecia show no indication of intelligence or even of choice, reacting to everything in the same manner, if they react at all. They have no power of adapting their actions to their needs. Chance is the main factor in bringing the Paramecium into proper conditions and giving it food, and if the chances are not favorable the animals must soon die. This agrees perfectly with the facts observed in cultures of these and similar animals. So long as the conditions are exactly right—bacterial zoogl&oelig;a covering everything, so that the Paramecia can not miss it, and the chemical condition of the water entirely favorable—the animals swarm and multiply by thousands. A slight change occurs in the conditions, and soon scarcely a Paramecium is to be found, though a few hours previously the water was milky with them. As in the case of plants, the proper conditions are the chief requisite for growth and multiplication;

these infusoria appear and disappear in the culture jar about as the lower algæ do. The power of movement, regulated in the simple manner above described, is correlated with the fact that, unlike plants, they live upon solid food (bacteria) and are therefore more likely to get this food if they can move about here and there. But the bacteria must be abundant in any case, for the Paramecia have no power of searching for them, or of choosing them rather than any other substance.

In future work it is hoped to determine how far the results gained on Paramecium are applicable to the Protozoa as a class, as well as to extend these researches to higher groups, building upon the foundation obtained by a study of these lowest organisms. In this way it is hoped that the laws which govern the movements and migrations of animals, the causes of their appearance and disappearance at certain places or under given conditions, and in fact much of their relations to the conditions surrounding them in the lake, may in time be made out. It is the belief of the writer that this is the most direct and certain way of unraveling the complicated network of relations which make up the life of the lake.

In addition to the study of the reactions of the animals above summarized, some faunistic work was carried on. An examination was made of the waters on and about South Bass Island, with the purpose of determining the abundance and general character of the Protozoan fauna. The swampy waters of this region were found to swarm with Protozoa of all sorts, offering unlimited supplies of material for work on the group in experimental or other lines. Unfortunately, the literature was not at hand for complete identification of all the species observed, so that critical systematic work, of the sort done on the Rotatoria, could not be carried on for the Protozoa. Only those could be positively identified that agreed completely with species described in the standard works on the Protozoa—Leidy's *Rhizopoda*, Kent's *Manual of the Infusoria*, Bütschli's *Protozoen*, Eyferth's *Die einfachsten Lebensformen des Thier-und Pflanzenreiches*, Blochmann's *Die mikroskopische Thierwelt des Süsswassers*, Pritchard's *Infusoria*, Ehrenberg's *Die Infusionsthierchen als volkommene Organismen*, etc.

The following list therefore contains the names of such species only as could be fully identified, and comprises thus but a fraction of the Protozoan fauna of the region. It is given in order to show something of the character of the abundant Protozoan fauna of these waters, as well as to point out forms that are of especial interest as favorable objects for investigation. Especial attention was paid to forms which from their size, or from the possibility of securing them in great abundance, promise to be particularly favorable for experimental work.

LIST OF SOME OF THE PROTOZOA FOUND IN THE WATERS ABOUT SOUTH BASS ISLAND, IN LAKE ERIE, DURING THE SUMMER OF 1898.

The waters examined were the same as those mentioned in the account of the Rotatoria. The list includes 68 species, distributed among the different groups as follows: Rhizopoda 13, Heliozoa 1, Mastigophora 21, Ciliata 32, Suctoria 1.

RHIZOPODA.

1. **Amœba proteus** Leidy. Taken in a water-bottle collection from the upper 3 feet of the surface of Lake Erie, 1 mile west of South Bass Island; also on *Elodea* from East Harbor and in the swamps on South Bass Island.
2. **Amœba villosa** Wallich. In the water-bottle collection from the upper 3 feet of the water of Lake Erie, 1 mile west of South Bass Island.
3. **Amœba radiosa** Ehrenberg. In the water-bottle collection from the upper 3 feet of the water of Lake Erie, 1 mile west of South Bass Island; also on *Elodea* from East Harbor, Lake Erie.
4. **Pamphagus hyalinus** Ehr. In surface towings in Put-in Bay Harbor, Lake Erie.
5. **Cochliopodium bilimbosum** Auerbach. In water-bottle collection from upper 3 feet of water of Lake Erie, 1 mile west of South Bass Island; also on *Elodea* from East Harbor, Lake Erie.
6. **Difflugia lobostoma** Leidy. In towings in Put-in Bay Harbor, Lake Erie; also from East Swamp on South Bass Island.
7. **Difflugia corona** Wallich. In surface towings from Put-in Bay Harbor, Lake Erie; also from East Harbor, Lake Erie; from Portage River, Ohio, and land-locked pools on Starve Island.
8. **Difflugia globulosa** Duj. In water-bottle collection from the surface 3 feet of Lake Erie, 1 mile west of South Bass Island; from surface towings in Put-in Bay Harbor; from East Harbor, and from the swamps on South Bass Island.
9. **Difflugia constricta** Ehr. In *Utricularia* from Portage River, Ohio.
10. **Difflugia pyriformis** Perty. In the swamp near the fish-hatchery on South Bass Island; in *Utricularia* from Portage River, Ohio.
11. **Arcella vulgaris** Ehr. In surface towings in Put-in Bay Harbor, Lake Erie; in East Harbor on *Elodea;* in the swamps on South Bass Island; from Portage River, Ohio.
12. **Centropyxis aculeata** Stein. In swamps on South Bass Island.
13. **Euglypha alveolata** Duj. Swamps on South Bass Island.

HELIOZOA.

14. **Acanthocystis chætophora** Schrank. In plankton haul from Lake Erie, just west of South Bass Island; on *Elodea* from East Harbor, Lake Erie.

MASTIGOPHORA.

15. **Oikomonas termo** J. Clark. Abundant on floating floccose material on the surface of Lake Erie, northeast of North Bass Island.
16. **Anthophysa vegetans** Müller. On *Elodea* from East Harbor, Lake Erie; from swamp on South Bass Island.
17. **Dinobryon sertularia** Ehr. On vegetation from East Harbor, Lake Erie.
18. **Euglena viridis** Ehr. In water-bottle collection from upper 3 feet of Lake Erie, taken 1 mile west of South Bass Island. Abundant in swampy parts of East Harbor, Lake Erie, and in the swamps on South Bass Island.
19. **Euglena spirogyra** Ehr. East Swamp, South Bass Island; Portage River, Ohio.
20. **Euglena oxyuris** Schm. East Swamp, South Bass Island.
21. **Amblyophis viridis** Ehr. East Swamp, South Bass Island.
22. **Colacium steinii** Kent. On *Diaptomus* sp. in surface towings from Lake Erie.
23. **Colacium vesiculosum** Ehr. On *Cyclops* in towings taken 2¼ miles north of Kelley Island, in Lake Erie; on *Polyarthra platyptera* and various crustaceans in swamps on South Bass Island.
24. **Trachelomonas hispida** Perty. In East Swamp, on South Bass Island; from Portage River, Ohio.
25. **Trachelomonas volvocina** Ehr. Amid filamentous algæ from East Harbor, Lake Erie; from Portage River, Ohio; from swamps on South Bass Island.

26. **Trachelomonas aspera** Ehr. Swamp on South Bass Island.
27. **Trachelomonas armata** Ehr. In aquatic vegetation from East Harbor, Lake Erie, and from East Swamp on South Bass Island.
28. **Phacus longicaudus** Ehr. From East Harbor, Lake Erie, and from swamps on South Bass Island.
29. **Phacus triqueter** Ehr. East Harbor, Lake Erie, and East Swamp, South Bass Island.
30. **Astasia trichophora** Ehr. On *Elodea* from East Harbor, Lake Erie.
31. **Entosiphon sulcatum** Duj. In jar of decaying *Nelumbo lutea* from East Harbor, Lake Erie.
32. **Synura uvella** Ehr. East Swamp, South Bass Island.
33. **Chilomonas paramecium** Ehr. Abundant in decaying water-plants from any part of Lake Erie or the swamps on South Bass Island. This is one of the species that can always be procured in unlimited quantities at any time.
34. **Monosiga steinii** Kent. On stems of *Epistylis plicatilis* from East Swamp, South Bass Island.
35. **Peridinium tabulatum** Ehr. In water-bottle collection from upper 3 feet of Lake Erie, 1 mile west of South Bass Island; also in aquatic plants from East Harbor, Lake Erie, and from the swamps on South Bass Island.

CILIATA.

36. **Trachelocerca olor** O. F. M. In *Utricularia* from Portage River, Ohio.
37. **Coleps hirtus** Ehr. Swamp near fish-hatchery on South Bass Island.
38. **Amphileptus meleagris** Ehr. In aquatic plants from East Harbor, Lake Erie.
39. **Amphileptus margaritifer** Ehr. In aquatic vegetation from Put-in Bay Harbor and East Harbor, Lake Erie.
40. **Lionotus fasciola** Ehr. In aquatic plants from East Harbor, Lake Erie.
41. **Loxophyllum meleagris** Ehr. On *Myriophyllum* from East Harbor, Lake Erie.
42. **Trachelius ovum** Ehr. On *Utricularia* from Portage River, Ohio.
43. **Dileptus anser** O. F. M. On aquatic plants from Put-in Bay Harbor, Lake Erie, and the swamps near the fish-hatchery on South Bass Island.
44. **Nassula ornata** Ehr. East Harbor, Lake Erie; Portage River, Ohio; East Swamp, South Bass Island.
45. **Glaucoma scintillans** Ehr. Common in cultures of decaying lake plants.
46. **Colpidium cucullus** Schrank. Abundant in infusions of decaying *Ceratophyllum* from the bottom of Put-in Bay Harbor, Lake Erie.
47. **Paramecium caudatum** Ehr. Abundant in cultures of decaying lake plants from the bottom of Put-in Bay Harbor, Lake Erie. See pp. 105–110 for an account of the laws of the movements of this animal.
48. **Urocentrum turbo** O. F. M. Abundant in decaying *Ceratophyllum* from the bottom of Put-in Bay Harbor, Lake Erie; also in water from East Swamp on South Bass Island.
49. **Cyclidium glaucoma** Ehr. In jar of decaying *Nelumbo lutea* from East Harbor, Lake Erie; many.
50. **Spirostomum ambiguum** Ehr. East Harbor, Lake Erie, and the swamps on South Bass Island.
51. **Bursaria truncatella** Müller. This enormous infusorian was common in the swamp near the fish-hatchery on South Bass Island.
52. **Stentor cæruleus** Ehr. In aquatic vegetation from East Harbor, Lake Erie, from Portage River, Ohio, and the swamps on South Bass Island.
53. **Stentor igneus** Ehr. In *Elodea* from East Harbor, Lake Erie.
54. **Strombidium turbo** C. and L. In decaying *Nitella* from East Harbor, Lake Erie.
55. **Halteria grandinella** Müller. Many in *Utricularia* from Portage River, Ohio.
56. **Tintinnopsis cylindrica** Daday. An empty shell of what appears to be this species, recently described by Daday, was taken in the water-bottle collection from the upper 3 feet of Lake Erie, 1 mile west of South Bass Island.
57. **Codonella cratera** Leidy. In towings from Put-in Bay Harbor, Lake Erie. This seems to be the same as the European *Codonella lacustris*, but Leidy's name has the priority.
58. **Holosticha mystacea** Stein. In the water-bottle collection from the upper 3 feet of Lake Erie, 1 mile west of South Bass Island.
59. **Uroleptus musculus** Müller. Few in decaying *Nitella* from East Harbor, Lake Erie.
60. **Oxytricha fallax** Stein. Common in decaying *Ceratophyllum* from the bottom of Put-in Bay Harbor, Lake Erie.

61. **Trichodina pediculus** Ehr. On *Diaptomus* from towings in Put-in Bay Harbor; on *Hydra* from East Harbor, Lake Erie.

62. **Vorticella convallaria** L. Very abundant on algæ from East Swamp, South Bass Island.

63. **Vorticella chlorostigma** Ehr. Forming large green patches visible to the naked eye, on the vegetation from East Swamp, South Bass Island.

64. **Vorticella rhabdostyloides** Kellicott. Common on *Anabæna* in towings from Lake Erie.

65. **Zoothamnium arbuscula** Ehr. In surface towings in Put-in Bay Harbor, Lake Erie, attached to floating matter.

66. **Epistylis plicatilis** Ehr. Abundant on *Chara* from East Swamp, South Bass Island, in company with *Megalotrocha alboflavicans*.

67. **Vaginicola crystallina** Ehr. On aquatic plants from East Swamp, South Bass Island. What seems the same form is often found on *Fragillaria* in towings from Lake Erie; these specimens are always much smaller, however.

SUCTORIA.

68. **Acineta mystacina** Ehr. On floating floccose material taken with the tow net in Put-in Bay Harbor, Lake Erie.

While the fauna inhabiting the plants of the bottom and about the shores of this part of Lake Erie is very rich in Protozoa, both in the number of species and of individuals, the open waters of the lake contain very few. Though 22 species are included in the list, as taken from the waters of the lake away from shore, most of these were present in very small numbers, and none were abundant. The species of the foregoing list found in the open waters of the lake, and on that account apparently to be considered limnetic, are the following:

Amœba proteus.
Amœba villosa.
Amœba radiosa.
Pamphagus hyalinus.
Cochliopodium bilimbosum.
Difflugia corona.
Difflugia globulosa.
Arcella vulgaris.
Acanthocystis chætophora.
Oikomonas termo.
Euglena viridis.
Colacium steinii.
Colacium vesiculosum.
Peridinium tabulatum.
Tintinnopsis cylindrica.
Codonella cratera.
Holosticha mystacea.
Trichodina pediculus.
Vorticella rhabdostyloides.
Zoothamnium arbuscula.
Vaginicola crystallina (?).
Acineta mystacina.

This list includes a number of species not usually recorded from open-lake waters; these are chiefly due to Professor Reighard's collections with the water bottle, which were made as follows: A large corked bottle was sunk in the lake to the desired depth, the cork pulled from the mouth, and the water allowed to fill the bottle. The water thus secured was then filtered, so as to prevent the escape of even the most minute organisms. Collections were thus made from the open lake 1 mile from any land, where the water was 6 fathoms deep. Water was taken from the surface layer not more than 3 feet below the surface. Collections so made contained regularly a number of minute Protozoa not usually accounted limnetic, namely:

Amœba proteus.
Amœba villosa.
Amœba radiosa.
Cochliopodium bilimbosum.
Difflugia globulosa.
Euglena viridis (once).
Peridinium tabulatum.
Tintinnopsis cylindrica (only once).
Holosticha mystacea.

The list is remarkable especially for the three species of *Amœba* and one of *Cochliopodium*. These rhizopods are very minute, and would be lost by the usual methods of collecting. Continued thorough plankton work of the sort carried on by Professor Reighard may show that these are proper members of the limnetic fauna.

Difflugia globulosa was one of the very commonest limnetic forms in all sorts of collections from the open lake.

The passive limnetic forms included in the preceding list are:

Oikomonas termo, on floating floccose material.
Colacium steinii, on *Diaptomus*.
Colacium vesiculosum, on *Cyclops*.
Vorticella rhabdostyloides, on *Anabæna*.
Zoothamnium arbuscula, on floating material.
Vaginicola crystallina (?) on *Fragillaria*.
Acineta mystacina, on floating floccose material.

The following species may be noted as of special interest because of their fitness as objects of investigation in experimental or other lines:

Chilomonas paramecium is a flagellate form that can always be procured in unlimited numbers by simply allowing the aquatic plants to decay in jars. The necessity for large numbers in carrying on experimental work needs no emphasis. The ease with which a Protozoan can be cultivated in the laboratory is almost the most important element in its availability for investigation.

The species of *Euglena*, *Phacus*, and *Trachelomonas* are always to be had in large numbers from East Swamp, South Bass Island.

Trachelius ovum, *Dileptus anser*, and *Nassula ornata* are ciliates which are valuable for certain sorts of work on account of their large size. The same is true to a more pronounced degree of *Spirostomum ambiguum*, and especially of *Bursaria truncatella*. The latter is an enormous creature for a unicellular animal, being a millimeter or more in diameter. It could thus be handled in the same individual way as many of the large Metazoa. This animal was always procurable in small numbers from the swamp near the fish-hatchery on South Bass Island. Doubtless a little experimentation would discover a means of cultivating them in large numbers. Perhaps there is no other Protozoan that would be so favorable an object for an investigation into the effects of localized stimuli and into the question of the localization of functions in the Protozoan body or related problems.

Other ciliates that could always be procured in large numbers are *Glaucoma scintillans*, *Colpidium cucullus*, *Paramecium caudatum*, *Urocentrum turbo*, *Cyclidium glaucoma*, and *Vorticella convallaria*.

For Rhizopoda, three species of *Difflugia*—*D. globulosa*, *D. lobostoma*, and *D. corona*—are particularly abundant and might be used for work on this group. Cultures properly managed usually resulted in obtaining large numbers of various species of *Amœba*.

Species of *Volvox*, *Eudorina*, *Pandorina*, etc., swarm in East Swamp, South Bass Island; they are not included in the foregoing list. A study of the physiology of these creatures, transitional as they are between Protozoa and Metazoa, promises much of interest.

DARTMOUTH COLLEGE, *Hanover*, *N. H.*, *May 25*, *1899*.

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CHAPTER 2

SENESCENCE AND DEATH IN PROTOZOA AND INVERTEBRATES

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1. PROBLEMS IN THE STUDY OF PROTOZOA

The questions which arise in the study of ageing and death in the Protozoa are mainly the following:

Does life in single cells involve decline, senescence, death? Does to live mean eventually to wear out, and to accumulate waste products that produce decline and death, even when it occurs in but a single cell?

Or is the life of single cells so carried on that wear is repaired as it occurs and waste products eliminated as they are produced, so that their life may continue without end? Are senescence and death phenomena that have taken origin only as organisms became multicellular and differentiated? Are they the consequence of division of labor, specialization, and interdependence among the cells of a multicellular organism, as many have held? What is the nature of the processes of repair that underlie the long-continued life of many unicellular organisms?

In view of the nature of the life cycle of the Protozoa, the age problem here has a two-fold bearing:

(1) The individual is a free cell, which performs all the fundamental operations of life. Does ageing play a rôle in the life of such a single individual?

(2) More important is the following. The single cellular individual as a rule does not as such die; instead it grows and divides into two individuals. This continues, so that chains of free individuals are produced, stretching for many cell generations. These are interrupted at long intervals by the union of two individuals in sexual reproduction. After this, the chain of free individuals continues. In relation to this, the question arises: Does the chain of successive living cells, through the processes of living, gradually become senescent in its later links, so that it declines and finally dies, unless something intervenes to save it?

These two aspects of living in free cells will be considered separately.

2. LIFE OF INDIVIDUAL PROTOZOAN

The single individual is produced by the division into two of an earlier individual. The parent has a typical differentiated structure, in many cases with typically arranged motor appendages (figure 6). The typical structure is necessarily altered and disordered by the division of the individual, so that the new individuals produced are not like the parent. But developmental processes occur by which the new individuals assume the same typical structure that was present in the parent. This development involves an extensive reorganization of the body. In cases in which the parent has complexly arranged appendages, these appendages are commonly absorbed and disappear during fission, while at the same time many other bodily differentiations disappear. The typical structure and appendages are then developed anew in each of the new individuals. The details of this reorganization differ in different species; it appears to be less complete in species having a less highly differentiated structure.

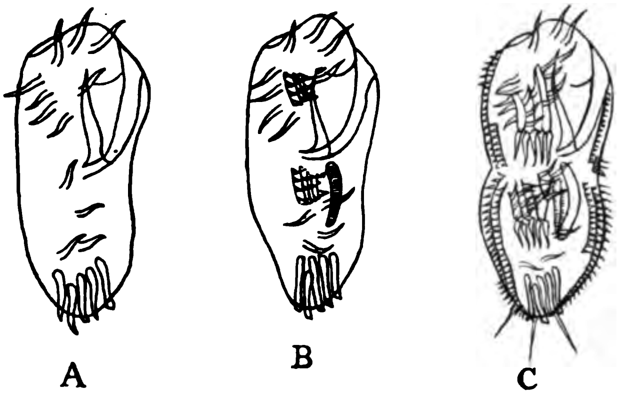

FIG. 6. Division with reorganization in the infusorian *Stylonychia*, after Wallengren (1901). (*A*) Parent before reproduction. (*B*) Appearance of two groups of small projections that are to form the appendages of the two offspring. (*C*) Division: the two groups of embryonic appendages are spreading out to take their final positions, while the old appendages have not yet disappeared.

This reorganization involves the nucleus of the cell, as well as the cell body. In the higher Protozoa,—the ciliate infusoria,—the nucleus is in two parts (originally derived from one). One is the very large macronucleus, the other the minute micronucleus (see fig. 7). It is the large macronucleus that participates in metabolism, growth and other activities of the cell, while the micronucleus is a reserve part, having little relation to the cellular processes except to maintain its own existence, and to produce new macronuclei.

At fission, both nuclei divide. During the process the macronucleus is in many cases known to be extensively reorganized. In some cases a portion of the macronucleus is eliminated and dissolved (fig. 8). In other cases a visible "reorganization zone" begins at a definite spot and traverses the entire nucleus, changing its structure (fig. 9). At the same time it seems probable, as has been suggested, that certain materials are eliminated, perhaps in a liquid condition.

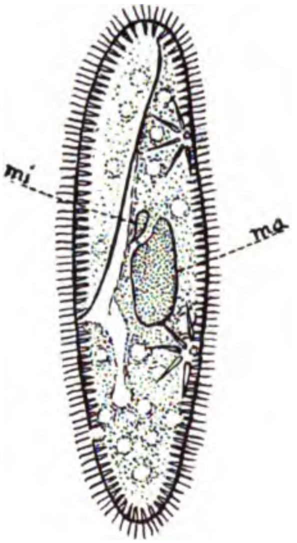

FIG. 7. The infusorian *Paramecium caudatum*, after Wenrich (1928), from Kalmus (1931), showing the large macronucleus (*ma*) and the small micronucleus [Stylonychia] (*mi*).

For accounts of such reorganization phenomena the following recent papers may be consulted: Kidder, 1933; Calkins, 1934; Kidder and Diller, 1934; Tittler, 1935; Summers, 1935. An excellent illustrated and detailed account of these and other matters relating to ageing and rejuvenescence in Protozoa will be found in Calkins' *Biology of the Protozoa* (2nd edition, 1933).

Thus after fission the newly produced individuals have been completely reorganized, both inwardly and outwardly; they are "young" as compared with the parent. Certain physiological changes as the individual recedes from youth have been revealed by studies of regeneraion. But these are the reverse of what might at first be anticipated, revealing the peculiar nature of individual life in these organisms. Calkins (1911) found that in *Uronychia* there is no regeneration if parts are removed in "young" individuals immediately after fission, and very little if they are removed later during "adult" life. But if in "old" individuals that are getting ready to divide, parts are removed, they are fully regenerated. The end of the individual life is here not death, but division into two, and this is preceded by an increase of growth energy instead of by senescence. However, such greater regenerative power just before division appears to be absent in some species (in *Spathidium*, according to Moore, 1924).

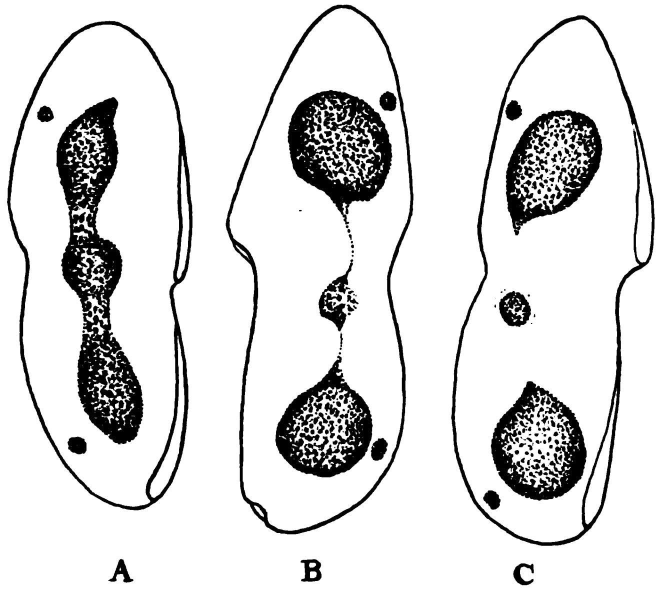

Fig. 8. Division of the macronucleus in *Ancistruma*, with extrusion of a central portion which is later absorbed and disappears. The small round bodies above and below are the micronuclei. After Kidder (1933).

Under some unfavorable conditions the individual cell may undergo a reorganization without division. This occurs as a rule (but not exclusively) in connection with encystment. Conditions that induce encystment and reorganization are, in different cases, the following: mechanical injuries to the cell, drying, lack of food, increase of salt content in the water, the presence of excretion products, and the like.

The first step in such a reorganization is a dedifferentiation of the cytoplasmic structures. The conspicuous buccal groove disappears, the motor appendages are absorbed, and the cell takes a spherical form, appearing as an undifferentiated mass containing the nuclei. Usually this spherical mass secretes a wall about itself, becoming thus a cyst, which is resistant to drying and to other injurious conditions. Dedifferentiation in the large and complex unicellular form *Bursaria* is described in detail by Lund (1917).

Within the cyst thus formed there occurs in many cases a reorganization of the nuclear apparatus. In its most pronounced form, this includes the breaking up and dissolution of the macronucleus and its replacement by a portion of the micronucleus. The latter divides, one part enlarging to become a macronucleus, another part remaining in reserve as a micronucleus. Such a reorganization of the nucleus is known in many cases; it is called *endomixis*. In some cases no such pronounced nuclear reorganization occurs in the cyst. The detailed nuclear changes during encystment are in need of extensive study.

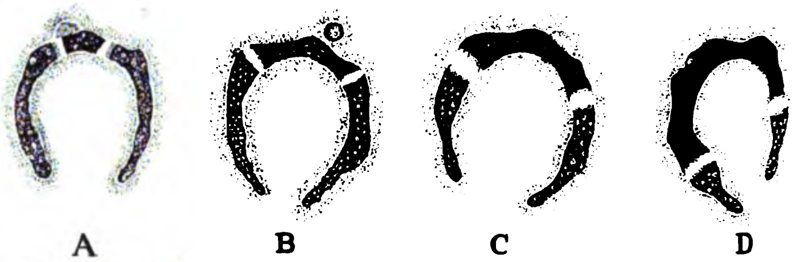

FIG. 9. Successive stages in the reorganization of the horse-shoe shaped macronucleus at division in *Aspidisca lynceus*, after Summers (1935). Before division two "reorganization bands" appear near the middle of the horse-shoe; these traverse the horse-shoe in each direction to its tips, inducing a thorough reorganization of the nucleus.

Under favorable conditions the undifferentiated cyst is awakened to active life. Redifferentiation occurs; the typical structures and appendages are again produced, the cell escapes from within the cyst wall and resumes its free existence. In cases in which before encystment the vitality was low, it is found that the reorganized individuals emerging from the cysts have a heightened vitality and reproductive power, as compared with the situation before encystment (Calkins, 1915; Beers, 1930). Encystment may occur at times when the vitality is high, being induced by unfavorable environmental conditions; in such cases encystment does not increase vitality.

The life of the individual cell as such is normally terminated by its vision into two cells. Hartmann (1928) has shown that by repeatedly tting off a part of the cell (in Ameba and Stentor) division may be ppressed. Individuals on which removal of a part is thus practiced regular intervals may live indefinitely without division. Hartmann us kept an individual Ameba alive for four months without division. appears that cutting off a piece of the body induces reorganization, ith the same effect as results from cell division.

3. AGEING OF PROTOZOA THROUGH MANY GENERATIONS

As shown in the foregoing section, reproduction by fission involves eorganization and development. The new individuals produced are hysiologically young as compared with the parent individual.

But the question remains whether the rejuvenescence so produced lly restores the pristine condition. The chain of living cells extends or hundreds, thousands, of generations. Are the later members of the hain as physiologically young as the earlier members? Or do the ater members become senescent and finally die, unless something ntervenes to save them? And by what sort of intervention may they e saved?

To obtain factual answers to these questions an astonishing amount f labor has been expended. The present state of knowledge will be ummed up in a series of numbered sections.

1. When the chains of living cells are kept under observation in the aboratory, so that their fate can be determined, as has been done with many species of Protozoa, it is found that as a rule, after some hundreds of generations they do decline. Fission and other life processes become slower, resistance decreases, functions are imperfectly performed. Varied structural changes occur. Frequently the protoplasm becomes opaque; the cell body is swollen, or in other cases wastes away. Structural differentiations become less marked; appendages may be lost. Nuclear abnormalities occur; at times the micronucleus disappears. The organisms give a picture of senescence, finally extending to death. Figure 10 shows three stages in these changes as observed by Maupas (1888).

The curve of declining vitality is well represented by the graph of daily fission rate, which for any given biotype appears to be a good measure of vitality. A typical graph is shown in figure 11. A large number of such graphs for different species, are given by Jennings 1929.

The period during which the animals continue to multiply, before declining to death, varies greatly. It usually includes some hundred of generations. Such a declining curve of *Paramecium caudatum*, a studied by Calkins (1904) covered 659 days, during which there wer 742 generations.

2. Such declining stocks are in some cases visibly rescued from deat and restored to high vitality by the intervention of sexual reproduc tion,—the union of individuals in conjugation.

Whether sexual union does in fact result in rejuvenescence was lon a subject of controversy. When conjugation occurs at periods whe

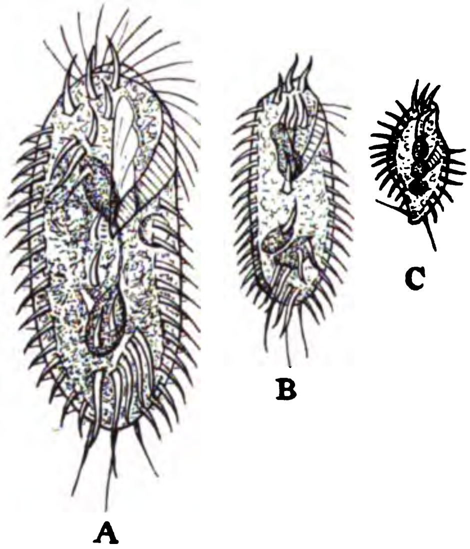

FIG. 10. Three successive stages (drawn to the same scale) in the decline and degeneration of *Stylonychia pustulata*, after Maupas (1888).

the vitality has not been lowered, it does not result in increased vitality. Moreover, conjugation has many other aspects; it results in producing new combinations of the genes; and it is a complex process, which may take place in an imperfect or abnormal way. In consequence, it often results in an actual lowering of vitality; it may even result in abnormality and death. It has now been fully demonstrated however that in certain species, if conjugation occurs at periods of lowered vitality, it results in increased vitality, in rejuvenescence. This was demonstrated by Calkins (1919) for *Uroleptus*, and by Woodruff and Spencer (1921) for *Spathidium*. After conjugation, in these cases, the lowered urve of vitality rises, the life processes proceed again rapidly and ficiently, and death is averted.

How sexual union produced this effect was long a mystery; it gave se to mystical theories. The explanation now seems clear; it will be t forth in later paragraphs.

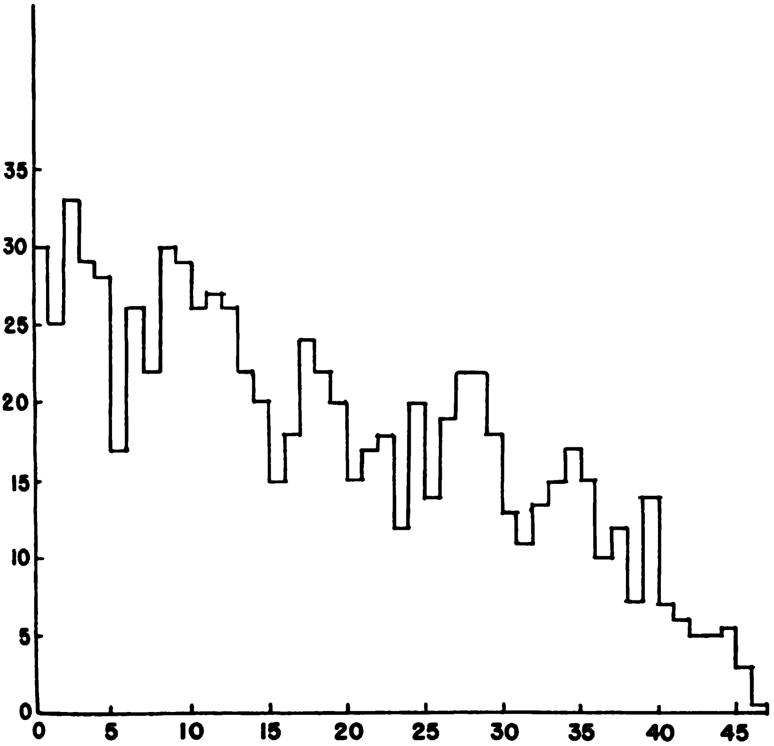

Fig. 11. Graph of the fission rate in successive ten-day periods in isolation cultures of *Pleurotricha lanceolata*, in the experiments of Baitsell (1914). The horizontal scale shows the number of successive 10-day periods, the vertical scale the number of fissions in each period. The fission rate declines (with many fluctuations) till the culture ends in death.

3. It has now been shown that there exist stocks or species of Protozoa which may continue indefinitely to reproduce by fission, without senescence or decline—the consecutive chain of cells continuing endlessly to multiply at a high level of vitality; and this without intervention of sexual union.

This was demonstrated by Woodruff (1926) in *Paramecium aurelia;*

the organisms were kept in uninterrupted vegetative multiplication for years, and for thousands of generations. It was similarly demonstrated for *Paramecium caudatum* by Metalnikov (1924), by Hartmann (1917) for *Eudorina*, by Belar (1924) for *Actinophrys*, and by other workers for other species of Protozoa.

Thus in these species the constitution of the organisms is such that the life processes can go on indefinitely. Regulatory processes, repair of wear and elimination of waste keep step with the other processes of life. One of the major questions appears to be answered by these observations. Senescence and death of the cell are not inevitable accompaniments or results of living.

4. But the deeper significance of these facts was brought into question by discovery of the nature of some of the regulatory processes which make possible this uninterrupted living. In the free cells that have been most studied in connection with these questions,—the ciliate infusoria—the nucleus, as before set forth, is in two parts. There is a large physiologically active macronucleus, and a minute reserve, the micronucleus. If the macronucleus is removed, life processes soon stop; but they may continue for generations in the absence of the micronucleus. If the major processes of living wear and use up the nuclear material, it is upon the macronucleus that this exhaustion must fall.

It was discovered by Woodruff and Erdmann (1914) that in *Paramecium aurelia* and *caudatum* there occur at intervals of many generations of active life a replacement of the entire active macronucleus from a part of the reserve micronucleus. This is the same process (endomixis) mentioned on an earlier page as occurring during encystment. But it was now found to occur in these species during active life, without interruption of the processes of vegetative reproduction. The macronucleus breaks up and fades away, the micronucleus divides and a part of it enlarges to take the place and functions of the macronucleus.

It has further been shown that this replacement of the worn macronucleus from the unworn micronucleus is effective in keeping up or restoring the vitality of the organism. If endomixis is omitted for long periods the organisms decline in vitality, resistance is decreased, and finally, if endomixis does not occur, they die. This was first observed by Woodruff (1917), and has been extensively confirmed in the laboratory of the writer. After endomixis has been long postponed, the fission rate falls, and the fall continues, becoming progressively greater the longer endomixis is held off, till death occurs (Jennings and Sonneborn, 1936). If after the fission rate has notably fallen, endomixis occurs, with its substitution of a new macronucleus for the old one, the fission rate in many cases is at once restored to the usual level, and the chain of cells lives far beyond the period at which it would have died. Normally in these species endomixis occurs before there has been a detectable fall in vitality, so that decline is entirely prevented. A delicate test gives evidence that in *Paramecium aurelia* the vitality is directly proportional to the recency of endomixis. This test is presented by the fact that the undergoing of the endomictic process is itself a severe ordeal, so that individuals of lowered vitality may die during or in consequence of it. The greater the period since the last endomixis, the greater the proportion of individuals that die at or after the occurrence of the next endomictic process (unpublished results of Pierson and Gelber).

The meaning of the occurrence of endomixis, with its renewal of vitality, appears to be as follows. An essential part of the cell, the nucleus, is indeed exhausted or poisoned in the process of living, so that life can be continued only by the substitution for this worn organ of a nucleus which has not partaken of the active life of the cell. In these species at least it appears to be this substitution of a reserve for a worn out part that makes it possible for life to keep indefinitely in progress. The necessary repair and elimination, it appears, are not carried out as part of the ordinary continuous physiological processes, but at intervals exhausted parts must be rejected and unexhausted parts substituted, such substitution occurring within the single cell itself. In place of the death of the entire cell there is the dissolution and replacement of the macronucleus.

This gave the clue to the restoration of lowered vitality by sexual union. In this process too the macronucleus breaks up and dissolves, and its place is taken by a part of the reserve. The substitution has the same effect in warding off senescence and renewing vitality, whether it occurs in connection with sexual reproduction or apart from it. In some of the Ciliata, beside the replacement of the macronucleus at sexual reunion, there is a visible reorganization of the entire cell. Appendages and other visible structures are lost and developed anew.

With these discoveries the balance seemed to incline again toward the inevitability of exhaustion through full active living, although reserve parts, it appears, can live unexhausted as a result of not participating in the major activities of life. One becomes inclined to suspect that the keeping in store of fresh unexhausted parts, whether they are entire cells as in higher organisms, or parts of cells as in these Protozoa, is the secret of continued life.

5. But another step in discovery brought again into question the general validity of such a conclusion. Certain stocks or species of Protozoa, it was found, can continue to live and multiply indefinitely, although in them there is no separation of the nucleus into active and reserve portions, and hence no replacement of the former by the latter. This is the case in Eudorina (Hartmann, 1921), and in Actinophyrys (Belar, 1924). Furthermore, in many species of ciliate infusoria, in which there is such a separation of active and reserve parts, there is no substitution of the reserve for the active part during the active life of the organisms, but only at encystment. Yet some of these species can live indefinitely in an active condition. And in certain species in which normally there exist macronucleus and micronucleus, at times stocks are found in which there is no separate micronucleus. Such a condition commonly occurs in the final stages of senescence, when the organisms are predestined to early death. But Dawson (1919) found a stock of *Oxytricha hymenostoma* in which the micronucleus was missing, yet vitality was high, and though vitality declined in isolation cultures, in mass cultures these infusoria lacking a micronucleus would live indefinitely.

Thus it appeared that in these species the substitution of a reserve for the active nucleus is not necessary. The balance seemed to swing again away from the conclusion that exhaustion and senescence are inevitable unless such substitution occurs.

But the last word on this matter has not been said. As set forth earlier, in the account of the life of the individual, at cell division parts of the active macronucleus are eliminated, in some species of infusoria. At division into two, a third piece of the nucleus is formed, and this is dissolved and disappears. In other cases, as already mentioned, a visible reorganization of the macronucleus occurs, which is not obviously accompanied by elimination of parts (unless the eliminated material is in liquid condition). Such phenomena are commonly interpreted as cases of elimination of exhausted portions of the nucleus, with presumably their replacement from reserve parts; or at least as processes of reorganization by which exhausted parts are renewed (see especially Calkins, 1934). They are of extreme interest, though it is much to be regretted that there appears no prospect of making an experimental test of the assumption that they actually result in a renewal of vitality.

All this leaves open the possibility that there are in all continuously living cells processes of substitution occurring on so minute a scale that they are difficult to detect or to test, and that this is the secret of the power of living indefinitely. Yet to conclude that these processes are so minute and so nearly continuous, is practically equivalent to the conclusion that the ordinary processes of life include repair and elimination so effective that life can continue indefinitely without exhaustion; to the conclusion that senescence and death are not in all free cells inevitable consequences of living,—though in some they are.

6. But another set of observations is important in relation to this matter of senescence. In the same species or stocks which under favorable conditions may live indefinitely without senescence there occurs under less favorable conditions a slow decline in vitality as generations pass. This is true both in those stocks in which substitution of unworn for worn parts is known to occur, and in those in which it is not. The effect of the unfavorable conditions cannot be perceived in one generation; often not in ten generations. But they apparently slow down repair and elimination, and after many, perhaps hundreds, of generations they produce senescence and final death. Decline in some of these stocks is checked and in large measure remedied by substitution of the reserve for the active nucleus, but in time even this fails; *Paramecium caudatum* and *Paramecium aurelia*, with their periodic endomixis, eventually under poor conditions become old and die.

Thus the cell constitution in many Protozoa is such that under certain conditions life may continue indefinitely; while under other conditions it may continue for a great number of generations, but eventually yields to senescence and death.

7. Finally, there exist also stocks in the Protozoa in which senescence and ultimate death are unavoidable, occurring under all conditions, including the best.

Such stocks are frequently produced, in Paramecium, at sexual reproduction. In the Protozoa, as in higher organisms, sexual reproduction is a process of producing many diverse genetic constitutions. Some of these genetic constitutions are such as may continue to live long, or even indefinitely, when the conditions are good. But others are of such a sort that the lines of descent to which they give origin may live for a number of generations, many or few, then they inevitably decline and die.

Many such short-lived stocks have been found to arise at conjugation in *Paramecium aurelia*; some of these are described by Jennings, Raffel, Lynch and Sonneborn (1932). Some of these stocks live for but a few generations after conjugation, producing a small number of descendants, which all die. Others continue to multiply for 10 to 40 generations or more, giving rise to a large population; but after a time all the members of the population decline and die. In some cases such a stock gives rise to a few lines that continue long, while the great majority of the lines are predestined to early death, as in the stock 11a, described in the paper just referred to.

Thus after a stock of Protozoa has gone through conjugation there is a great process of selective elimination occurring among their descendants. A considerable proportion of the descendants of diverse genetic constitution so produced cannot continue to live indefinitely; after a small or large number of generations they decline and die, leaving only the more vigorous lines of descent.

This occurrence of stocks that are predestined by their genetic constitution to decline and death after multiplying for a number of generations, is a matter not to be neglected in a consideration of senescence and death in free cells. Ageing and death from intrinsic causes is not foreign to organisms that are single cells, as is often asserted. Death from intrinsic causes by no means owes its first origin to the evolution of multicellular organisms. It is a widespread and abundant phenomenon in unicellular organisms.

Summarizing the relations of free living cells to senescence and death, as we have set them forth above, we find many gradations, many diversities of constitution. Some free cells are so constituted that they are predestined to decline and death after a number of generations. Some are so constituted that decline occurs, but this is checked or reversed by substitution of reserve parts for those that are exhausted; they can live indefinitely, but are dependent on this substitution. In some the constitution is such that life and multiplication can continue indefinitely without visible substitution of a reserve nucleus for an exhausted one; but whether this is due to the continued substitution, on a minute scale, of reserve parts for those that are outworn cannot now be positively stated. This perfected condition, in which living itself includes continuously the necessary processes of repair and elimination, is found in some free cells, but not in all.

(Many of the matters discussed in the foregoing pages will be found more fully described or discussed in the present author's *Genetics of the Protozoa* (1929), and in Calkins' *The Biology of the Protozoa* (1933)).

4. INVERTEBRATES CONTRASTED WITH PROTOZOA

The invertebrates of course present a wide variety of conditions as to length of life, senescence and death. The length of life varies from the few days of the male rotifer to the sixty-six or more years of the actinian (Ashworth and Annandale, 1904). A summary of the chief known facts as to length of life and related matters is given by Korschelt (1917), and many important relations touching them are investigated and discussed in the writings of Pearl (1922, 1928). The present discussion will deal in the main with matters that touch the questions raised in the foregoing section on Protozoa, together with certain additional relations for which the invertebrates furnish favorable material. There are many parallels in the relations of the two groups to senescence and death.

In the invertebrates, as in the Protozoa, we find extensive if not universal use of the device of keeping certain parts of the organism out of the main current of the life activities, forming a reserve, which is substituted at intervals for worn or senescent parts of the active organism. In the multicellular organisms these reserves are distinct cells, not mere reserve portions of cells, as in the Protozoa. These reserve cells have the capability of developing and of taking on all the activities of the organism. They are best known to us as germ cells, but many organisms have other reserve cells, known as embryonic or regenerative cells, which remain dormant, but can be roused to active life by appropriate conditions. They play a great rôle in vegetative reproduction and in regeneration.

Besides substitution of reserve cells for exhausted or injured active ones, there may occur, as in the Protozoa, dedifferentiation and reorganization of differentiated cells, which thereupon take on new functions and differentiations. Such reorganization takes place readily in the partially differentiated cells of early embryonic periods, so that the "prospective fate" of the cells is changed. It becomes infrequent as adult conditions are reached. Different groups of the invertebrates differ greatly as to the readiness with which the "prospective fate" of the cells can thus be changed. In general it may be said that the extent and distribution of such reorganization of differentiated cells is not fully worked out.

The reserve cells known as germ cells are commonly aroused to full activity by the processes of sexual union. But the many cases of parthenogenesis, natural or artificial, show that they, like the regenerative cells, may be awakened to full activity independently of sexual union.

In the invertebrates, as in the Protozoa, the cells which take an active part in the life of the individual are subject as a rule to decline, to senescence. As we have seen in the Protozoa, such decline occurs in free cells as well as in those forming the differentiated multicellular body. In addition to the senescence of the individual cells, or in consequence of it, there is, as in the vertebrates, a general senescence of the entire body, culminating in death.

5. DETERMINATE AND INDETERMINATE GROWTH OF INVERTEBRATES

With relation to these matters there may be distinguished in the invertebrates certain different groups. On the one hand there are those having determinate growth. In these the body reaches a definite form and size, then ceases growing. The period of growth is commonly followed by a definite period of maturity, then of age, followed by death. Examples of these are the insects, and the rotifers. In another group growth is indeterminate; the form and size are not definitely fixed, and growth may continue for an indefinite period. Marked examples of these are many sponges, and the branching hydroids. Many invertebrates show mixed or intermediate conditions. Examples of these are some of the free-living flatworms, in which the individuals have a definite form and size, yet may grow indefinitely by the budding off of new individuals.

Invertebrates with determinate growth. In the most pronounced types of strictly determinate growth, as in the rotifers and the insects, embryonic or reserve cells may be lacking in adult life, except for the germ cells. Such organisms are usually without the power of regenerating lost parts, and all the cells except the germ cell are subject to senescence and death.

In these organisms the individual life is commonly divided into definite limited periods, differing in their relation to development. It will be worth while to note the characteristics of these in the rotifer as an example of strictly determinate development.

In the rotifer there is first a brief period of cell division, lasting but a few hours. At this time are produced all the cells of the various bodily tissues. According to some observers, the number of cells in each tissue is very sharply defined and limited, so that the exact number of cells in the body can be given; thus Martini (1912) gives the number of cells in the body of *Hydatina senta* as 959. According to Shull (1918) there is some inconstancy in the number of cells in particular tissues, but the fact of definitely limited cell production remains. The body is formed on a pattern in which the number of cells in each part is extremely constant. Cell division occurs until the number of cells required for completing the pattern is supplied, then it ceases. At this time the cells are packed solidly together, and have not taken on their definitive forms, so that the individual is a minute nearly spherical mass of cells.

Upon the cessation of cell division, a period of growth and differentiation follows. Without increase in the number of cells, the various organs take on their adult forms, their cells differentiate, growth in size occurs, till the normal size and structure of the organisms are produced. Thereupon growth ceases. This period of growth lasts in the rotifers but a few hours.

Next follows a brief period of maturity, during which reproduction occurs. It lasts in most species but a few days, and is definitely limited, though it is not quite so uniform as the periods of cell division and of growth. The reproductive period varies in *Proales sordida* from 3 to 9 days (Jennings and Lynch, 1928).

Next follows a period of senescence, ending in death. The period of senescence is limited, like the others, though with a somewhat greater amount of variation. In *Proales sordida* it varies from 1 to 17 days. The total life in this animal falls within 23 days, divided as follows: cell division, one day; growth, one day; reproductive period 3 to 9 days; senescence 1 to 17 days.

As appears from the above, the different life periods, except possibly the last, are dedicated to certain definite functions, ceasing when those functions are accomplished, so that they are thereby sharply limited. The entire life proceeds upon a rather definite schedule, occupying but a few days. In many cases the organisms are so constituted that early decline and death forms one of their most definite characteristics. In the male of most rotifers there is no alimentary canal, so that life lasts but a few hours, and a similar situation occurs in other groups. Death at a particular time in the schedule appears as definite a characteristic of the species as form of body or limbs.

Such organisms have raised in certain minds the question whether in them senescence and death, like cell division, growth and reproduction. are not essentially functional; whether the time at which they occur has not become fixed by the same evolutionary processes that fix the color of eyes or form of limbs. Of this character was a famous theory of Weismann, holding that death at a certain period had been developed through natural selection, as an advantage to the species, rather than as an inevitable consequence of exhaustion through the wear and tear of living.

Invertebrates with indeterminate growth. In the invertebrates in which development is indeterminate, as in hydroids and some flatworms, life of the body appears to continue indefinitely, through continued branching, budding, or division. Yet the parts that are early formed and differentiated commonly become in time senescent and finally die. It is only the newly formed buds or branches that continue to live indefinitely,—through further budding or branching. A valuable detailed study of these phenomena in the flatworm Stenostomum is given by Sonneborn, 1930. In such organisms the senescence that occurs seems to be a true decline or "decay" resulting from wear or incomplete elimination of waste products.

In the formation of the "new" parts of the body, that continue to live, reserve or embryonic cells that have not taken part in the full life of the body are known to play a large rôle. When such embryonic cells are destroyed by radiation, new parts are not further produced; regeneration no longer occurs (Curtis, 1928). In some cases also dedifferentiation of differentiated cells plays a rôle; they take on anew the embryonic condition, later developing and differentiating anew. In many highly differentiated cells such restoration to the embryonic condition appears not possible. The extent to which dedifferentiation plays a rôle in these matters has not been fully elucidated.

6. GENETIC CONSTITUTION AND SENESCENCE

In any given species, particularly in those organisms having determinate growth, the length of life and the time of occurrence of senescence and death, depend (as in Protozoa) both on environmental conditions, and on the genetic constitution. Important investigations on both these aspects of the matter in invertebrates, we owe to Pearl and his co-workers (see Pearl, 1922, 1928, and Gonzalez, 1923). The rôle of genetic constitution in determining length of life they have demonstrated fully in the fruit fly, *Drosophila melanogaster*. Under given conditions, certain stocks, marked by visible structural characteristics, live much longer than others. Gonzalez (1923) found that while the normal "wild type" fruit fly lives on the average 39.5 days, a stock differing from this in having much reduced wings ("vestigial wings") has an average length of life of but 18.2 days. The vestigial stock differs from the wild type in but a single gene. Gonzalez shows also that changes in other single genes result in greatly altering the length of life. The average duration of life in days for a number of stocks differing in but a single gene (or in the case of "Quintuple" differing from the others in five genes) is given by Gonzalez (1923) as follows:

	days
Wild type	**39.47**
Black	**40.68**
Purple	**25.54**
Vestigial	**18.22**
Arc	**26.81**
Speck	**42.66**
Quintuple	**10.88**

As is well known, every gene (with seemingly a few exceptions) plays an essential rôle in the life and development of every cell of the body. It is not surprising therefore that changing single genes may so alter the cellular processes as to change the length of life.

When stocks differing in length of life are crossed, Pearl shows that in the descendants the length of life depends upon their genetic constitution. In the immediate offspring of the cross (F_1), the mean length of life is greater than in either parent stock, so that these offspring show "hybrid vigor." In a cross of a "type I" (mean life 44.2 days) with a "type IV" (mean life 14.1 days), the mean life in the immediate offspring was 51.5 days (Pearl 1922). In later generations of the descendants, groups with diverse lengths of life are formed, in proportions showing that inheritance of this character, like that of others, occurs in accordance with Mendelian laws.

In general it has been found that in Drosophila the mutated types tend to have a higher mortality rate, and a lower length of life than the unmutated or "wild" types,—almost any mutation in a gene tending to shorten life.

In invertebrates, as in man, there is much variation in the length of life among the individuals of a given species or race. If in a population the proportion of survivors is plotted at successive intervals, a curve of typical form is produced. Pearl brings out that in the invertebrates, so far as these matters have been studied, this survivorship or mortality curve is of the same form and type as in man. Pearl and Doering (1923) have illustrated this in detail for Drosophila and the rotifer, *Proales decipiens*, in comparison with man.

What are the causes of the variations in length of life, and the typical distribution of these variations shown in the survivorship curves? In a human population, both environmental and genetic factors are certainly involved, genetic differences among the individuals perhaps supplying the main cause of variation. In the rotifer, *Proales sordida* however, there is a similar mortality curve, although the individuals are all derived by uniparental reproduction from a single ancestor, so that genetic diversities based on varying distribution of the genes are not to be expected. The differences in duration of life possibly point to the existence of congenital diversities of a different type from those due to differences in distribution of genes; diversities hardly perceptible in other ways.

7. SUMMARY

Our present knowledge of ageing and death of protozoa and invertebrates indicates the importance of at least two factors.

The first of these is the substitution of reserve parts for those that become exhausted. Some protozoa are provided with both a large (macro) and a small (micro) nucleus. The latter functions as a reserve. In the invertebrates and in man a comparable intracellular reserve is not seen but there is an intraorganismal reserve of cells. These include the sex cells capable on union of producing another whole multicellular organism to take the place of the old one and of various relatively undifferentiated cells which by multiplication make good parts of the organism which may wear out. Among the latter are the reserves, being called upon as long as the individual lives, to produce new blood cells and epidermal cells of the skin.

The second is genetic or hereditary. There can be no doubt that in all animals from the single celled protozoa, through the invertebrates to man, the length of life is largely determined by inheritance. In fruit flies a single gene may make a great difference in length of life. In humans there are long and short lived families and further study will probably reveal the importance of genetic constitution as one factor in ageing.

REFERENCES

Ashworth, J. H., and Annandale, N. 1904. Observations on some aged specimens of Sagartia troglodytes, and on the duration of life in Coelenterates. Proc. Roy. Soc. Edinburgh, **25**, (pt. IV), 1–14.

Baitsell, G. A. 1914. Experiments on the reproduction of the hypotrichous infusoria. II. A study of the so-called life cycle in Oxytricha fallax and Pleurotricha lanceolata. Jour. Exp. Zool., **16**, 211–235.

Beers, C. D. 1930. Encystment and restoration in the ciliate Didinium nasutum. Jour. Exp. Zool., **56**, 193–208.

Belar, K. 1924. Untersuchungen an Actinophrys sol Ehrenberg. II. Beiträge zur Physiologie des Formwechsels. Arch. f. Protistenkunde, **48**, 371–434.

Calkins, G. N. 1904. Studies in the life history of Protozoa. IV. Death of the A series. Conclusions. Jour. Exp. Zool., **1**, 423–464.

——— 1911. Regeneration and cell division in Uronychia. Jour. Exp. Zool., **10**, 95-116.

——— 1915. Didinium nasutum. I. The life history. Jour. Exp. Zool., **19**, 225-241.

——— 1919. Uroleptus mobilis, Engelm. II. Renewal of vitality through conjugation. Jour. Exp. Zool., **29**, 121–156.

——— 1933. The Biology of the Protozoa. 2nd Ed., 667 pp., Philadelphia.

——— 1934. Factors controlling longevity in Protozoan protoplasm. Biol. Bull., **67**, 410–431.

Curtis, W. C. 1928. Old problems and a new technique. Science, **67**, 141–149.

Dawson, J. A. 1919. An experimental study of an amicronucleate Oxytricha. Jour. Exp. Zool., **29**, 473–513.

Gonzalez, B. M. 1923. Experimental studies on the duration of life. VIII. The influence upon duration of life of certain mutant genes of Drosophila melanogaster. Amer. Nat., **57**, 289–325.

Hartmann, M. 1917. Über die dauernde rein agame Züchtung von Eudorina elegans und ihre Bedeutung für das Befruchtungs- und Todproblem. Ber. d. preuss. Akad. d. Wiss., Phys.-Math. Klasse, 760–776.

——— 1921. Die dauernd agame Zucht von Eudorina elegans, experimentelle Beiträge zum Befruchtungs- und Todproblem. Arch. f. Protistenkunde, **43**, 223–286.

——— 1928. Über experimentelle Unsterblichkeit von Protozoen-Individuen. Ersatz der Fortpflanzung von Amoeba proteus durch fortgesetzte Regenerationen. Zool. Jahrb., Abt. f. allg. Zool. u. Physiol., **45**, 973–987.

Jennings, H. S. 1929. Genetics of the Protozoa. Bibliographia Genetica, **5**, 105–330.

Jennings, H. S., and Lynch, R. S. 1928. Age, mortality, fertility, and individual diversities in the rotifer Proales sordida Gosse. I and II. Jour. Exp. Zool., **50**, 345-407 and **51**, 339-381.

———, Raffel, D., Lynch, R. S., and Sonneborn, T. M. 1932. The diverse biotypes produced by conjugation within a clone of Paramecium aurelia. Jour. Exp. Zool., **62**, 363–408.

———, and Sonneborn, T. M. 1936. Relation of endomixis to vitality in Paramecium aurelia. C. R. XII^e Congr. Internat. de Zool., Lisbon, 416-420.

Kalmus, H. 1931. Paramecium, das Pantoffeltierchen. Jena: 188 pp.

Kidder, G. W. 1933. On the genus Ancistruma Strand (Ancistrum Maupas). I. Structure and division of A. mytili Quenn. and A. isseli Kahl. Biol. Bull., **64**, 1–20.

———, and Diller, W. F. 1934. Observations on the binary fission of four species of common free-living ciliates, with special reference to the macronuclear chromatin. Biol. Bull., **67**, 201–219.

Korschelt, E. 1917. Lebensdauer, Altern und Tod. Jena: 170 pp.

Lund, E. J. 1917. Reversibility of morphogenetic processes in Bursaria. Jour. Exp. Zool., **24**, 1–33.

Martini, E. 1912. Studien über die Konstanz histologischer Elemente. III. Hydatina senta. Zeitschr. f. wiss. Zool., **102**, 425–645.

Maupas, E. 1888. Recherches expérimentales sur la multiplication des infusoires ciliés. Arch. de. Zool. Exp. et Gén. (2), **6**, 165-277.

Metalnikov, S. 1924. Immortalité et rajeunessement dans la biologie moderne. 283 pp., Paris.

Moore, E. Lucile. 1924. Regeneration at various phases in the life-history of the infusorians Spathidium spathula and Blepharisma undulans. Jour. Exp. Zool., **39**, 249-316.

Pearl, R. 1922. The biology of death. 275 pp., Philadelphia and London.

——— 1928. The rate of living. 185 pp., New York.

——— and C. Doering. 1923. A comparison of the mortality of certain lower organisms with that of man. Science, **57**, 209-212.

Shull, A. F. 1918. Cell inconstancy in Hydatina senta. Jour. Morph. **30**, 455–464.

Sonneborn, T. M. 1930. Genetic studies on Stenostomum incaudatum (nov. spec.). I. Jour. Exp. Zool., **57**, 57-108.

Summers, F. M. 1935. The division and reorganization of the macronuclei of Aspidisca lynceus Müller, Diophrys appendiculata Stein, and Stylonychia pustulata Ehrbg. Arch. f. Protistenkunde, **85**, 173–208.

Tittler, I. A. 1935. Division, encystment and endomixis in Urostyla grandis. La Cellule, **44**, 189-218.

Wallengren, H. 1901. Zur Kenntniss des Neubildungs- und Resorptionsprocesses bei der Theilung der hypotrichen Infusorien. Zool. Jahrb., **15**, 1-38.

Wenrich, D. H. 1928. Eight well-defined species of Paramecium (Protozoa, Ciliata). Trans. Amer. Micr. Soc., **47**, 275–282.

Woodruff, L. L. 1917. Rhythms and endomixis in various races of Paramecium aurelia. Biol. Bull. **33**, 51-56.

——— 1926. Eleven thousand generations of Paramecium. Quart. Jour. Biol., **1**, 436–438.

———, and Erdmann, R. 1914. A normal periodic reorganization process without cell fusion in Paramecium. Jour. Exp. Zool., **17**, 425–518.

———, and Spencer H. 1921. The survival value of conjugation in the life history of Spathidium spathula. Proc. Soc. Exp. Biol. and Med., **18**, 303-304.

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SEX REACTION TYPES AND THEIR INTERRELATIONS IN PARAMECIUM BURSARIA. I

II. CLONES COLLECTED FROM NATURAL HABITATS

(6)

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SEX REACTION TYPES AND THEIR INTERRELATIONS IN PARAMECIUM BURSARIA. I

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Communicated February 1, 1938

The discovery by Sonneborn[1] of two sex reaction types in a race of *Paramecium aurelia* opened a new chapter in the genetics of the Ciliata. The present study shows that similar reaction types occur in *Paramecium bursaria*, but that in number and interrelationships they differ greatly from the situation in the race of *Paramecium aurelia* studied by Sonneborn and by Kimball.[2]

In the work on the cytology of conjugation in *Paramecium bursaria*, in this laboratory, Dr. T. T. Chen observed that conjugating pairs could be obtained from mixtures of two of his cultures, but not from either culture separately. This formed the starting point of the present investigation. Two clones, designated *l* and *m* were obtained from the cultures of Chen, and the work was extended to many other stocks. The clones *l* and *m* had been collected from a pond at Alexandria, Virginia.

The mating reaction that occurs when two appropriate clones of *Paramecium bursaria* are mixed—such clones as *l* and *m*—is of the rapid agglutinative character described by Sonneborn[1] in *Paramecium aurelia*, and by Moewus[3] for various flagellates. Within a few seconds after mixture the individuals have clotted together in small groups containing two to a dozen or more individuals. In strongly marked reactions these groups quickly coalesce into masses that contain hundreds of individuals and are visible to the naked eye. The animals remain clotted for a half hour to two hours; in the latter part of that period the clots begin to break up, and it is now found that many of the component individuals have become united in pairs. After about two hours only united pairs and single individuals may be found in the mixture.

The pairs are formed from one member of each of the two clones that were mixed. This is readily demonstrated by removal of the green color from individuals of one of the two clones. The usual strong green color of *Paramecium bursaria* is due to the presence in the body of many cells of a green alga. The color can be almost completely removed by inducing the animals to multiply rapidly in a rich nutritive medium, such as the algal-bacterial medium described by Raffel.[4] The contained algae multiply much less rapidly than the Paramecia, so that the later generations contain but a few scattering alga cells which hardly color them at all. In this condition *Paramecium bursaria* is practically as colorless as *Paramecium aurelia*. When one of the two clones is thus made white, the pairs in the mixture are found to consist always of one green individual and one white one. In the large clots also, green individuals are always in contact with white ones.

The reaction between two clones belonging to diverse reaction types occurs whenever the two clones are mixed, provided they are in the proper physiological condition. There is a period after conjugation, varying in length in different clones, in which the individuals will not mate. Further, mating does not occur when the individuals have recently been transferred to a rich nutritive medium and have grown very large and are rapidly dividing. The most favorable conditions for the mating reaction are furnished when, after such a period of rapid growth and division, the nutritive medium becomes poor and the animals are becoming thin and ceasing to divide. At such periods, if two clones of diverse type are mixed the clotting is strongly marked and many pairs are formed.

Different clones show marked differences in the readiness with which they are brought into the condition for clotting and conjugation. Some are refractory and can be induced to react only by bringing about the exact degree of nutritive decline that is most favorable to the mating reaction. Others react readily under all conditions except those of extreme plumpness and rapid fission resulting from rich nutritive conditions. The reaction occurs, however, only at certain periods of the day, diverse in different stocks, but excluding in all cases the evening hours, so far as observed. (These matters are to be dealt with separately.)

The clotting reaction above described occurs only when two clones of diverse sex reaction type are mixed. But in certain clones, perhaps in all, isolated pairing may occur between members of the same clone, if the clone is left for long periods in a condition of declining nutritive strength. If, for example, great numbers of individuals of a single clone are left for several days in a watch glass with a small amount of water, after some days isolated pairs may sometimes be found among them. The formation of such pairs is not accompanied by clotting, and is so rare that close study of a clone for months may yield no examples. Such exceptional pairs are left out of account here; they form no part of the usual picture of pairing. Their genetic consequences will be dealt with elsewhere.

In such clones as *l* and *m* we thus find two sex reaction types that are seemingly comparable to the plus and minus sex types of Chlamydomonas and other flagellates as described by Moewus,[3] and to types I and II of Paramecium aurelia (Sonneborn 1).

The matter was investigated further (1) by allowing *l* and *m* to conjugate together, producing many exconjugant clones, the sex reactions of which were examined, and (2) by collecting from natural habitats many stocks and studying their sex reactions. Results of the former line of work are presented in this paper.

Sex Reaction Types of Clones Descended from Exconjugants of Type l by Type m.—A large number of pairs were obtained by matings between the clones *l* and *m*, representing the two reaction types. From the exconjugants of these pairs clones were obtained, and the sex type of each was tested by examining its reactions against the two parental types. The design was to obtain if possible inheritance ratios for the two diverse types.

This purpose meets a serious obstacle in the great mortality among the exconjugant lines. Most of the exconjugants or their descendants die. This occurs even in the most favorable nutritive media and other conditions in which non-conjugants flourish, none dying. A large proportion of the exconjugants die without dividing. Others divide, but produce weak offspring, often small and abnormal, which die after a few generations. In the experiments, 142 pairs were isolated from *l* × *m*; these should yield 284 exconjugants, and as two lines were to be cultivated from each exconjugant, there were potentially 568 lines of descent. But out of these, only 26 formed clones that continued to multiply and so could be tested; thus but 4.6 per cent. And these included representatives of but 11 of the 142 pairs.

With so high a mortality the determination of inheritance ratios for the sex reaction types is not possible, but study of the 26 descendant clones nevertheless furnishes important information as to the sex reaction types and their nature.

The exconjugant lines were designated as follows. To each pair was given a number, and the two members of a pair were called *a* and *b*. From each member two lines of descent, derived from the first division of the exconjugant, were isolated; these were designated 1 and 2. Thus the pair 67 furnished the four lines of descent 67*a*1 and *a*2, 67*b*1 and *b*2. The 26 exconjugant lines were tested by mixing samples of them respectively with *l* and with *m*, to determine the sex type. As was expected, some reacted only with *l*, others only with *m*.

But the clone 67*a*1 was found to form pairs with both *l* and *m*, and on examination this was found to be the case with all the 4 clones descended from pair 67—*a*1, *a*2, *b*1 and *b*2. They reacted strongly, forming immediately large clots, and numerous later pairs, equally with *l* and with *m*.

It was at first suspected that such clones must have become differentiated into the two sex types, as *Paramecium aurelia* differentiates into two sex types at endomixis, according to Kimball.[2] But examination showed that this is not the case. Any single individual of 67*a*1 (or the other clones of 67) reacts with equal readiness with either *l* or *m*.

This is discovered as follows. A single individual of 67*a*1 is introduced with capillary pipette into a drop containing individuals of clone *l* only. The first individual of *l* with which it comes in contact clings to it and initiates the pairing reaction. While in this conidtion, before firm union, the pair are removed to a drop containing only individuals of *m*. In the transfer the two become separated, since they have not yet firmly united. Each then provokes a strong reaction with *m*; in a few seconds two small clots are formed. In one of these individuals of *m* are clinging to *l*, in the other to 67*a*1. It is demonstrated that the single individual of 67*a*1 reacts with equal readiness with type *l* or type *m*.

		1				2									3													4			
		m	C	23b1	23b2	l	B	3a1	5b1	15b1	28b1	107a1	107a2	88a1	5b2	67a1	67a2	67b1	67b2	88a2	88b1	88b2	97a1	97a2	97b1	97b2	169a2	1b1	169a1	169b1	169b2
1	m	−	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	C	−	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	23b1	−	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	23b2	−	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
2	l	+	+	+	+	−	−	−	−	−	−	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	B	+	+	+	+	−	−	−	−	−	−	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	3a1	+	+	+	+	−	−	−	−	−	−	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	5b1	+	+	+	+	−	−	−	−	−	−	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	15b1	+	+	+	+	−	−	−	−	−	−	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	28b1	+	+	+	+	−	−	−	−	−	−	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	107a1	+	+	+	+	−	−	−	−	−	−	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	107a2	+	+	+	+	−	−	−	−	−	−	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
	88a1	+	+	+	+	−	−	−	−	−	−	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
3	5b2	+	+	+	+	+	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	+	+	+	+
	67a1	+	+	+	+	+	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	+	+	+	+
	67a2	+	+	+	+	+	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	+	+	+	+
	67b1	+	+	+	+	+	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	+	+	+	+
	67b2	+	+	+	+	+	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	+	+	+	+
	88a2	+	+	+	+	+	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	+	+	+	+
	88b1	+	+	+	+	+	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	+	+	+	+
	88b2	+	+	+	+	+	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	+	+	+	+
	97a1	+	+	+	+	+	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	+	+	+	+
	97a2	+	+	+	+	+	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	+	+	+	+
	97b1	+	+	+	+	+	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	+	+	+	+
	97b2	+	+	+	+	+	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	+	+	+	+
	169a2	+	+	+	+	+	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	+	+	+	+
4	1b1	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	−	−	−
	169a1	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	−	−	−
	169b1	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	−	−	−
	169b2	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	−	−	−

TABLE 1

PARAMECIUM BURSARIA

Clones of the *l* — *m* group, including 26 clones derived from exconjugants of *l* × *m*. Classified table of matings, showing the four reaction types.

+ Form clots and pairs when mixed.

— Do not clot and pair.

Also, if a single individual of 67*a*1 is allowed to multiply, and some of its descendants are tested with *l*, others with *m*, they are found to react immediately with both.

Thus we have, besides *l* and *m*, a third reaction type, which unites with both *l* and *m*. We are not dealing with simply two reaction types, as was at first assumed.

In the course of the tests a fourth reaction type was found, exemplified by the clone 1*b*1. This forms clots and pairs, not only with *l* and with *m*, but also with 67*a*1 and other members of its type.

To determine the exact situation, each of the 26 clones descended from *l* and *m* was tested with every other, as well as with the parental clones *l* and *m*. In addition, two other clones, *B* and *C*, were found to interact with members of this group; these were included in the tests. The testing of each of the 30 clones with each other one involves ½ (30 × 29) or 435 tests. In each test, 50 to 100 or more individuals of each of the two clones to be tested are placed together in 2 or 3 drops of water and observed for clotting and pairing.

The results of these 435 tests are shown in table 1. As the table shows, the 30 clones (26 derived from *l* × *m*) fall into 4 diverse sex reaction types; there are indeed 4 types among the descendants of the two types *l* × *m*.

The first of these is the *m* type, containing but four clones, including two descended from *l* × *m*.

The second, the *l* type, includes nine clones; among them seven derived from the union of *l* and *m*.

The third, the 67*a*1 type, contains 13 clones, all derived from the union of *l* and *m*.

The fourth, the 1*b*1 type, contains 4 clones descended from *l* × *m*. Thus the 26 exconjugant clones derived from the union of *l* and *m* fall into four reaction types in the proportions 2:7:13:4.

The four types have the peculiar relation to each other that the members of each one clot and conjugate with the members of all the other three. The relations for the four types are shown in table 2, in which the plus sign indicates clotting and pairing in the mixture indicated.

TABLE 2

THE FOUR SEX REACTION TYPES AND THEIR INTERACTIONS, IN THE *l* - *m* GROUP

	l	*m*	67*a*1	1*b*1
l	−	+	+	+
m	+	−	+	+
67*a*1	+	+	−	+
1*b*1	+	+	+	−

Certain points with regard to inheritance may be gleaned from table 1. From the union of two parent types, *l* and *m*, we obtain 4 types; two of which (*m* and 1*b*1) are less abundant than the other two In some cases all four clones descended from a pair are of the same reaction type; this is the case in pairs 67 and 97. In other cases lines derived from the same pair, and even from the same exconjugant of a pair, belong to different reaction types. This is the case for example with 5*b*1 and 5*b*2, with 88*a*1 and 88*a*2, with 169*a*1 and 169*a*2. A segregation of the sex types thus occurs in some cases at the first division after conjugation, as is true in *P. aurelia* (Sonneborn[1]).

The clones produced from the exconjugants of *l* × *m* differ in many ways besides the differences in their sex types. There are characteristic differences in size, in form, in vigor and particularly in their readiness to react sexually. Some, like the parental clones, react quickly under almost any condition. In others, specially favorable conditions must be supplied, of the nature already mentioned, in order to make possible the characteristic reactions when they are placed with individuals of another type.

A discussion of the relation of these facts to the problem of sex differentiation is reserved until the sex reaction types of numerous stocks collected in nature have been presented.

[1] Sonneborn, T. M:, *Proc. Nat. Acad. Sci.*, **23**, 378–385 (1937).

[2] Kimball, R. F., *Ibid.*, **23**, 469–474 (1937).

[3] Moewus, F., *Arch. f. Protistenk*, **80**, 467–526 (1933).

[4] Raffel, D., *Biol. Bull.*, **58**, 293–312 (1931).

SEX REACTION TYPES AND THEIR INTERRELATIONS IN PARAMECIUM BURSARIA. II. CLONES COLLECTED FROM NATURAL HABITATS

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Twenty-seven clones of *Paramecium bursaria* were collected from diverse localities: four from near Alexandria, Virginia, twenty-three from the neighborhood of Baltimore, Maryland. After allowing the single individuals to multiply in the laboratory, the sex type of each clone was tested, originally by mixing with individuals of the two first-discovered types *l* and *m*, described in the foregoing paper.

The four Virginia clones, *l* and *m*, *B* and *C*, which all came from the same pond, were found to form but two reaction types, *B* being of the same type as *l*, *C* of the same type as *m*.

But unexpectedly, the clones from the region about Baltimore did not react with any of the Virginia types. As shown in the foregoing paper, two additional reaction types were developed from the two that originally occurred in the Virginia group. None of the 23 Maryland clones reacted with any of the four reaction types of the Virginia group. In mixtures of any Maryland clone with any Virginia clone, there is no clotting or pairing.

The twenty-three Maryland clones were further tested by making mixtures of each clone with each other one, giving rise thus to 253 mixtures. Five of the Maryland clones did not react with any others. Repeated mixtures of these with each of the other clones were made, but no clotting or pairing occurred under any conditions.

		5						6						7				8	9
		AR	Ff	Fg	Fj	Fm	S	Fa	Fb	j	T	U	V	Fd	Fh	Fk	Fp	Fl	Fo
5	AR	−	−	−	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+
	Ff	−	−	−	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+
	Fg	−	−	−	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+
	Fj	−	−	−	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+
	Fm	−	−	−	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+
	S	−	−	−	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+
6	Fa	+	+	+	+	+	+	−	−	−	−	−	−	+	+	+	+	+	+
	Fb	+	+	+	+	+	+	−	−	−	−	−	−	+	+	+	+	+	+
	j	+	+	+	+	+	+	−	−	−	−	−	−	+	+	+	+	+	+
	T	+	+	+	+	+	+	−	−	−	−	−	−	+	+	+	+	+	+
	U	+	+	+	+	+	+	−	−	−	−	−	−	+	+	+	+	+	+
	V	+	+	+	+	+	+	−	−	−	−	−	−	+	+	+	+	+	+
7	Fd	+	+	+	+	+	+	+	+	+	+	+	+	−	−	−	−	+	+
	Fh	+	+	+	+	+	+	+	+	+	+	+	+	−	−	−	−	+	+
	Fk	+	+	+	+	+	+	+	+	+	+	+	+	−	−	−	−	+	+
	Fp	+	+	+	+	+	+	+	+	+	+	+	+	−	−	−	−	+	+
8	Fl	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+
9	Fo	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−

TABLE 3

PARAMECIUM BURSARIA

Clones from natural habitats near Baltimore, Maryland, showing the five diverse sex reaction types (numbered 5 to 9).

+ Form clots and pairs when mixed.

− Do not clot and pair.

The other 18 Maryland clones reacted in some of the mixtures. The reactions fall into a system which is shown in table 3. Every clone conjugates with the members of certain other clones, and refuses to conjugate with those of certain others.

As table 3 shows, the 18 Maryland clones fall into five diverse reaction types, in addition to the four reaction types shown by the clones of the Virginia group. The Maryland types are numbered 5 to 9 in table 3. The reaction type 5 includes 6 clones, the members of which do not conjugate together, but do conjugate with the members of the four other types of the Maryland group. Type 6 again contains six clones, which do not interconjugate, but do conjugate with the members of the five other types of this group. The seventh type consists of four clones, while types 8 and 9 consist each of a single clone. Members of each type clot and conjugate with the members of every other type, but not with members of their own type.

	1	2	3	4	5	6	7	8	9
1	−	+	+	+	−	−	−	−	−
2	+	−	+	+	−	−	−	−	−
3	+	+	−	+	−	−	−	−	−
4	+	+	+	−	−	−	−	−	−
5	−	−	−	−	−	+	+	+	+
6	−	−	−	−	+	−	+	+	+
7	−	−	−	−	+	+	−	+	+
8	−	−	−	−	+	+	+	−	+
9	−	−	−	−	+	+	+	+	−

TABLE 4

PARAMECIUM BURSARIA

The nine sex reaction types thus far discovered, with their interactions, showing the two groups. Types 1 to 4 form the first or Virginia group, types 5 to 9 the second or Maryland group.

+ Form clots and pairs when mixed.

− Do not clot or pair.

Thus the nine diverse sex reaction types so far observed in *Paramecium bursaria* fall into two independent groups, the members of which do not conjugate with each other. The nine diverse types and their interrelations, with the fact that they fall into two groups, are shown in table 4.

The relations of the nine diverse reaction types to the localities from which they come are of interest. The four reaction types (numbers 1 to 4) which constitute the Virginia group all come from the same pond at Alexandria, Virginia, or are derived by descent from those that came from this pond. The five remaining types, constituting the Maryland group, come from two ponds near Baltimore. Type 5 consists of six clones from a small pond ("Falls Road Pond") a few miles north of Baltimore. Other clones from this pond are found in the other four types, 6 to 9. Type 6 contains four clones (*j*, *T*, *U*, *V*) from the central pond of the Botanical Garden of Johns Hopkins University, and in addition two clones from the Falls Road Pond. The remaining three reaction types are all composed of clones from the Falls Road Pond. Thus the Falls Road Pond contained members of all the five reaction types of the Maryland group.

In view of the two diverse reaction groups formed respectively by the clones from Virginia on the one hand, by those from Maryland on the other, examination of clones from other localities will be of interest; the author expects to study such, as they can be obtained. The conditions thus far found suggest that there may be many local races, differing in sex reaction type, and not reacting with races from distant localities.

A deeper understanding of these types must wait upon a study, with adequate numbers, of their inheritance in crosses and in self-fertilization. Such a study is in progress.

What is the relation of the findings in this and the preceding paper to the differentiation of sexes? In contradistinction to the flagellates investigated by Moewus, such ciliates as Paramecium are diploid and each of the two mates furnishes a migratory pronucleus and a stationary pronucleus, the former playing the part of a male gamete, the latter the part of a female gamete. There is thus a reciprocal fertilization, and each of the two mates gives rise to a line of descendants. The two mates are thus equivalent in these respects; each is comparable to a hermaphrodite rather than to a male or female. Nevertheless the two mates are differentiated in relation to the mating reaction, and the studies thus far made point to an interesting series of steps leading toward the development of two definite types comparable to two sexes. The observations on *Paramecium bursaria* here set forth suggest nothing of the sort. They show the existence of many diverse types, the members of any one of which do not conjugate together (under ordinary conditions), but do conjugate with members of various other types. But in *Paramecium aurelia*, as shown by Sonneborn and by Kimball, in a given race there are two and only two reaction types, and any single individual may, by endomixis, produce these two but no others. Yet each type still plays in other respects the part of a hermaphrodite. In the Vorticellidae the differentiation has gone farther; the two types are morphologically diverse, and one plays essentially the rôle of a female, the other that of a male. Seemingly two definite sexes have arisen by gradual specialization among an originally large number of diverse reaction types.

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SOCIAL LIFE AND INTERRELATIONSHIPS IN CERTAIN PROTOZOA

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Protozoa are organisms consisting of a single cell. Such interest as their social life has is derived from that fact. The social phenomena that they show are not due to the organization and interaction of many cells of different types, such as are found in higher organisms, but are part of the life of the single cells.

Social life involves the reactions of individuals as such to each other; involves discriminatory reactions to different individuals. Such reactions occur in the Protozoa mainly in relation to reproduction, particularly in the mating that is a part of biparental reproduction.

In certain Protozoa the different individuals are differentiated into diverse sex types. These react to each other differentially, as do the individuals of different sex in higher animals. In the Protozoa the sex types differ only physiologically, in ways that condition their behavior, but not structurally. Such differential sex reactions are known in the flagellate Infusoria (Chlamydomonas, Polytoma) mainly through the investigations of Moewus, Hartmann and other workers in Germany and in the Ciliate Infusoria (Paramecium, Euplotes) through the work of Sonneborn, Jennings, Chen, Kimball, Gilman and other investigators in America. The differential sex behavior gives rise to rather complex social systems.

In most species and varieties that have been investigated there are two sex types, which show behavior reactions that are similar to those shown by the two sexes in higher organisms. This is true so far as studied for the Flagellates and some of the Ciliates, including Paramecium aurelia and P. caudatum. But in certain species, as in Paramecium bursaria and in Euplotes patella, there may be in any variety more than two sex types. Details concerning these are given in later paragraphs.

Social behavior, with differential reactions, are found mainly in the relations of individuals belonging to different sex types. Individuals of the same sex type do not react sexually together, do not conjugate. But individuals of different sex type, if they belong to the same variety, readily react sexually and conjugate.

The sexual reactions are of a striking character. When all the individuals present are of the same sex type, there is no reaction; the individuals remain scattered and separate and do not conjugate. But if with these are mingled individuals of a different sex type, there is at once a remarkable change in behavior. The individuals of the different sex types adhere together, forming large clots. Each clot may include, under favorable conditions, hundreds of adhering individuals. At first the clots are small, but they increase rapidly in size. Later, after three or four hours or more, the large clots of adhering individuals break into smaller groups. Many of the separating components are seen to be united in pairs, by contact of their oral surfaces; others are again single, as at the beginning. The individuals that are united in pairs do not separate, but become closely adherent. They continue their union (conjugation) until complex processes have occurred in their nuclei, including repeated division and exchange of parts of the nuclei and chromosomes. This requires many hours; often in Paramecium bursaria the pairs remain united for 36 hours or more.[1]

In their obvious features the sexual reactions just described are like those seen in the minute Flagellata, and in most other Ciliata. But in the Flagellata the two individuals of a pair do not separate, but unite completely to form a zygote which later divides to produce new individuals. In all the Ciliata the two members of a pair separate after exchange of chromosomes, and each divides, producing many vegetative descendants.

In the Ciliata, such as Paramecium bursaria, the animals are large enough so that individuals can be followed separately and the details of behavior determined. The individuals of different sex types are not pulled together or attracted from a distance. After the sex types are mingled the animals continue to swim about in the same way as when all are of one sex type. The movements are no more rapid or "excited", nor modified in direction than when but one sex type is present. But the usual movements of many individuals in many directions in a small quantity of fluid soon bring some of them into accidental contact. It is then that the reaction occurs. The two that have come in contact, if they belong to different sex types, stick together as if their surfaces were covered with an adhesive material. There is no definite movement of reaction that brings about their contact. On the contrary, each of the two individuals is seen at first to try (as it were) to continue moving just as before, but upon contact both are suddenly stopped by their adhesion; or one drags the other backwards or obliquely, against the motion of its cilia. Only if the two anterior ends happen to lie in the same direction, as is rarely the case, do the two swim forward together. Any parts of the body that come in contact adhere. Two individuals may come in contact by their oral or aboral surfaces or by their rear ends, or in any irregular way; thereupon they adhere and consequently move irregularly, as a result of the uncoordinated action of the cilia of the two.

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[1] Photographs of various stages in the sexual behavior described have been published by Jennings, *American Naturalist*, Vol. 73, 1939, page 387; *Biological Symposia*, Vol 1, 1940, page 119 and *Genetics*, Vol. 24, 1939, page 204, also by Sonneborn in Calkins and Summers' *Protozoa in Biological Research*, 1941, page 691.

Two individuals thus adhering irregularly flounder about and may soon come in contact with other individuals of one or the other sex types. Such third individuals adhere irregularly to the original ones (always the adhesion is to the individual that is of different sex type from the new one); thus new individuals are continually added, till large masses or clots are formed, containing often a hundred individuals or more. In these clots the individuals are in irregular contact by any parts of their bodies, though always any two that adhere are of different sex types.

The large clots thus formed within three or four minutes after the mixture of the two sex types commonly remain seemingly unchanged for one to several hours, then begin to break up. In the meantime, by shiftings within the mass, some pairs of individuals have come into contact by their oral surfaces and these surfaces then become firmly united. Such pairs remain united until the complex nuclear changes and exchanges have occurred. After these are finished the two members of the pair (in the Ciliates) separate and each may continue its independent life, each having now a new combination of chromosomes formed from parts of the chromosome store of the two individuals that conjugated. In favorable cases each divides repeatedly, giving rise to a large number of individuals all having the same chromosome combinations and hence the same inherited characteristics. Such a group of individuals, derived by fission from the same ancestral ex-conjugant and all having the same characteristics, is known as a clone.

Thus the essential processes in the mating reaction (conjugation) are: (1) the normal active movements, bringing individuals of different sex type into accidental contact; (2) adhesion of those that are of different sex types, when they come in contact; (3) the gradual shifting of such individuals as are in irregular contact until some of them reach the normal mating position with oral surfaces united, axes parallel and anterior ends in the same direction; (4) more complete union of the oral surfaces; (5) internal changes, including division of nuclei and exchange of parts of them along with their contained chromosomes; (6) separation of the ex-conjugants and (7) their division into many individuals, forming a clone.

The account just given, based primarily on Paramecium bursaria, is typical for mature stocks in other species of that genus. In Euplotes patella the conjugation occurs between two individuals without the antecedent formation of clots of many adhering individuals. In the flagellates, which are very minute, clots are formed like those in Paramecium, but the individuals of different mating types are said to be attracted together from a distance.

But many conditions alter the sexual behavior, or prevent the reactions entirely, rendering the behavior in a high degree discriminatory. Certain types of individuals react together in the way described, while others do not. The principal categories of conditions that alter the behavior are the following:

1. If all the individuals present are of the same sex type they do not react sexually at all; do not clot together, nor unite in pairs. (Rare exceptions to this have been described for certain species under special conditions; these are mentioned later).

2. Individuals of young clones, produced by the division of ex-conjugants (individuals that have just completed conjugation), are immature and do not react sexually at all; do not conjugate until they have reached a certain age.

3. If the individuals present belong to different varieties of the species, or to different species they do not react sexually, do not conjugate, whatever the sex types to which they belong.

Each of these three categories presents points of interest, some of which are set forth in later paragraphs But before proceeding with these, certain other kinds of social behavior must be mentioned: behavior which does not as a rule lead to conjugation. There are two of these other types of behavior.

1. One of these other types shows itself in association of pairs of individuals in coordinated motion, yet without adhesion and usually without leading to conjugation. Two individuals accidentally touch each other. They then move off in coordinated motion, side by side, at least the cilia in contact, but the bodies not adhering together. They swim thus in a graceful spiral for a few seconds, remaining in contact, then they may separate, or they may continue in contact, swimming together for a longer period. Often after thus moving together for a time they separate; then soon come together again, and this alternation may be repeated a number of times, until as a rule they finally separate and each goes its own way.

Yet if the two individuls are different sex types of the same variety the coordinated motion in contact may finally result in adhesion, union, and completed conjugation. In some species of Ciliates indeed (Euplotes patella) this appears to be the usual or exclusive method of bringing about conjugation, for in such species the preliminary formation of clotted masses does not occur.

But such coordinated swimming together does not usually lead to conjugation, for it may occur in two individuals that are of the same mating type and hence do not conjugate. It may also occur in individuals that belong to different varieties, and in individuals one or both of which are immature. In all such cases conjugation does not result.

In this behavior discrimination of individuals is seen only in the fact that in some cases (where the individuals belong to different mating types of the same variety) adhesion and conjugation result, while in other cases they do not. Viewed as a whole, this type of behavior might be considered exploratory in functions: a process of finding appropriate mates.[2]

2. The second type of reaction between individuals that does not lead to conjugation is seen in the toxic or lethal reactions between individuals of certain varieties, described by Sonneborn in Paramecium aurelia. When individuals of one variety are brought into close proximity with those of a certain other, the individuals of one of the varieties are injured: they spin about on the long axis, swell, and soon die. "Two stocks produce toxic substances that kill animals of other stocks. The two toxic substances produce characteristically different effects prior to death, and each acts differently on the three groups of stocks of P. aurelia and on certain other species of Paramecium. These substances play an important role in natural selection, for, when a stock that produces a toxic substance and a susceptible stock are grown together the latter is soon completely killed off," (Sonneborn, *Science,* November 25, 1938, Vol. 88, p. 503).

Returning to the typical behavior that leads to conjugation, including preliminary adhesion into clots, the three different categories of modification and discrimination mentioned on a previous page show further matters of interest as follows:

1. Diversity of Sex Type—As before seen the completed sexual reactions occur as a rule only between members of different sex types of the same variety. The adhesion of surfaces which is a prominent feature of these reactions is a reciprocal matter; it does not occur unless the individuals are of different sex types. It is a result of the difference between the two surfaces.

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[2] Figures of this behavior have been published by Jennings, *Genetics,* Vol. 24, 1939, Figure 2, page 207.

Moewus has elucidated the nature of these differences in the flagellates. He finds that the two different sex types in Chlamydomonas are diverse in the possession of certain organic chemicals, or "sex-stuffs." These belong to the group of carotinoids. One of the sex types (which Moewus calls the female) has a sex-stuff that is a carotinoid known as cis-crocetin dimethylester, while in the other sex type ("male"), the sex-stuff is a modification of the same material, known as trans-crocetin dimethylester. By mixing these two in varied proportions, sex-stuffs characteristic of the different varieties in Chlamydomonas are produced. In the ciliates no such analysis of the sex-stuffs has been made.

In any variety of the flagellates, so far as examined, there are two sex types, so that the situation is analogous to that in the higher organisms, where there are two sexes. In the Ciliates a similar situation is found in Paramecium aurelia, and seemingly in Paramecium caudatum; there are just two sex types in any variety. But in certain other Ciliates there are, in a single variety, more than two sex types. In Euplotes patella there were found six diverse sex types in the stock examined by Kimball. In Paramecium bursaria one variety has eight sex types; two others have four sex types each, while in a fourth variety there are but two sex types. In those cases in which there are several sex types, members of any one sex type may conjugate with individuals of any other sex type of the variety.

Thus in Variety I of Paramecium bursaria there are four sex types, which have been designated A, B, C, and D. Individuals of the sex type A may react sexually with individuals of any of the other three sex types, so that pairs containing an individual of A may have the constitution AB or AC or AD. Similarly any individual of B, C, or D react sexually with any individual of the other three types. Thus from this variety with its four sex types there may be formed pairs of six different constitutions, namely, AB, AC, AD, BC, BD and CD. Variety II in this same species has eight different sex types designated respectively as E, F, G, H, J, K, L, M. Since members of any sex type may conjugate with members of any of the other seven, consideration shows that from the eight sex types pairs of 28 different constitutions may be formed, from EF to LM. In Variety III of Paramecium bursaria there are again four sex types, N, O, P, and Q; from these again pairs of 6 diverse constitutions may be formed. In Variety IV of the same species there are but two sex types, R and S, so that all pairs formed are of the same constitution, RS. Thus in the four varieties of Paramecium bursaria the number of pairs of diverse constitutions that may be formed is 6+28+6+1, or 41.

Any two sex types, as A and B, are distinguishable by the fact that either may conjugate with all other types of the variety but not with its own type. Thus A will conjugate with B, C and D, but not with others that are A; B will conjugate with A, C and D, but not with others that are B, and so on.

All members of the same clone (derived by fission from a single exconjugant), are found, on becoming mature, to belong to the same sex type. Thus in Variety I of Paramecium bursaria, some clones are all A, others are all B, others C, others D. This fact, that all members of a clone are alike in sex type (as well as in other genetic characteristics) makes it convenient to employ the clone as the unit in experimentation and in discourse. Clones may be mated with other clones of different sex type; a clone refuses to mate with other clones of its same sex type, and so on. The clone thus corresponds in many ways to the individual body of higher organisms. Both consist of many cells, though in the clone these cells are separate and may be scattered in space and time. Also the cells of the clone are usually all alike, while those of the body are functionally differentiated. But the clone as a unit is of a particular sex type, as the body is of a particular sex. Like the body, the clone is at first sexually immature; later it is mature; still later both as a rule become aged and finally die.

Thus any clone is of a definite sex type and when mature, it conjugates with a clone or clones of different sex type. But if no opportunity is given for conjugation with a clone of different sex type, the single clone differentiates into two diverse sex types, which thereupon conjugate together. In species in which there are but two sex types, the self-differentiation of a clone is of course into those two. In varieties which include more than two sex types, the differentiation of any clone produces always a particular pair of sex types. In Variety I of Paramecium bursaria some clones that are of sex types A differentiate into A and B; others into A and C, others into A and D. In Paramecium aurelia Sonneborn has shown that the self-differentiation of a clone into two sex types as a rule takes place as a result of certain nuclear processes, known as autogamy (at endomixis). But in a few cases it has been established that in this same species such self-differentiation into two sex types may occur during ordinary vegetative reproduction, without the occurrence of endomixis (Kimball). In some species, as in Paramecium bursaria, self-differentiation of a clone occurs very infrequently at seemingly irregular intervals of usually a year or more. In others, as in some varieties of Paramecium aurelia, it occurs at frequent intervals, of but about two to four weeks.

In consequence of the self-differentiation of the clone into two sex types, the members of a single clone may conjugate together, as if they belonged to two distinct clones. The two sex types into which a clone thus differentiates remain distinct; if they are cultivated separately the descendants of the two remain of the diverse sex types into which the clone had differentiated.

The conjugation that occurs within a self-differentiated clone thus still conforms to the rule that it is members of two diverse sex types that unite in conjugation. But there have been described a few cases in which under special conditions members of the same sex types unite sexually, though in most such cases conjugation is not completed. Moewus observed in a species of Chlamydomonas such incomplete conjugation between members of the same sex type. In this species, from a dense culture in which all are of one sex type the individuals (cells) are removed by centrifuging or filtration, leaving only the fluid. If now individuals of the other sex type are treated with this filtered fluid, they are induced to clot together, just as occurs when individuals of both sex types are present. But there is no final complete union of the individuals, such as occurs when the two are of different sex type; soon they separate.

Similarly Sonneborn found that when individuals of Paramecium aurelia belonging to two sex types are mixed, for a short time some individuals that are of the same type clot together, though in these cases as in those described by Moewus, completed conjugation does not occur between individuals of the same sex type.

In Euplotes patella, however, Kimball found that in mixtures of two sex types, at times completed conjugation occurs between what were at the beginning members of the same sex type. It is possible that a temporary change in sex type had occurred though this appears improbable. The change could be only temporary, since in such cases when the two individuals that are in the preliminary stages of conjugation are cultivated separately, the two give rise to clones that are still of the same original sex type (Kimball).

Conditions parallel to those just set forth for Paramecium aurelia and Euplotes patella have recently been discovered by T. T. Chen in Paramecium bursaria. Studies of these phenomena will be published by Chen.

Thus in most cases conjugation occurs only between members of different sex types. But previous contact with members of a different sex type, or with fluid in which these have lived, may induce a temporary clinging together of members of the same sex type, or in some cases may induce completed conjugation.

We have seen in previous paragraphs that change of sex type may occur at self-differentiation of a clone, through endomixis or otherwise. Changes of sex type occur also as a result of conjugation. The two members of a conjugating pair are of course (at least as a rule) of different sex type; each one when it multiplies without conjugation produces a clone of its own distinctive sex type. As a result of conjugation, one or both may change in sex type, so as to yield after conjugation descendants that are of different sex type from those produced before conjugation. In Paramecium aurelia, in which but two sex types exist in any variety, the two ex-conjugants of a pair may both yield clones of sex type I, or both may yield clones of sex type II, or one may yield sex type I, the other sex type II. In Paramecium bursaria both ex-conjugants of any pair as a rule produce clones of the same sex type. But different pairs from the conjugation of the same two clones yield in many cases, different sex types. For example, in Variety I with its four sex types, A, B, C and D, if many individuals of a clone of sex type A conjugate with members of a clone of sex type D, some of the pairs (which are originally all AD) produce descendants that are all of sex type A, others, descendants that are all of sex type D, while a few pairs produce descendants that are of sex type B, a few others, descendants that are of sex type C. The general rule in this species is that most of the pairs produce descendants that are in sex type the same as one or other of the two clones that conjugated, while a few yield descendants of different sex type from either parental conjugant. From conjugants of but two of the four types may be produced descendants that are of three or of all the four types that exist.

2. Immaturity and Maturity—The reaction between individuals is determined or modified not only by differences in sex type, as set forth above, but by differences in age of clones. After conjugation, in the Ciliates the two ex-conjugants separate and each divides vegetatively many times, so as to produce a large number of separate individuals, constituting a clone. Usually for some period, short or long, all the individuals of the young clones are sexually immature; they do not react sexually nor conjugate with any other members of the variety, whether mature or not, and whatever their sex type. The immature individuals do not differ visibly from mature ones except in this absence of sex reaction and of conjugation. The length of the period of sexual immaturity differs much in different species and even among clones of the same variety of the same species. In Paramecium bursaria the period of immaturity is usually long; observed immature periods have varied in different cases from twelve days to many

months. In Paramecium aurelia the immature period is short or nonexistent; it varies in different cases from zero to a week or ten days. In Paramecium bursaria it has been found that the length of the immature period depends to some extent on the nutritive conditions under which the individuals lived. Those that have been kept well-fed and in rapid multiplication become mature much earlier than members of the same ex-conjugant clone that have been subjected to unfavorable conditions such as reduce the rate of multiplication.

During the immature condition individuals may react to each other by the coordinated swimming in contact that was previously described, but they do not adhere together and do not conjugate. As maturity approaches some of the individuals of the clone begin to show a weak tendency to adhere when they touch. This period might be called adolescence; it may last for weeks and include many generations. The tendency toward conjugation becomes stronger, till in time the individuals show the strong and spectacular mating behavior that was before described. The new clone is now mature. It is not till maturity is reached that it is possible to discover to what sex type the clone belongs.

3. Non-interbreeding Groups or Varieties—The complexity of the social life in the Protozoa is greatly increased by the fact that given species have not only diverse sex types, which conjugate together, but include also, in cases adequately studied, groups of individuals that do not intercross, but remain sexually and genetically isolated. Each such group has its own set of sex types which conjugate together, but members of one group do not conjugate with members of other groups. Since members of such different groups do not intercross, the groups may be called varieties, though they may not be distinguishable by other characteristics.

In Paramecium aurelia Sonneborn has discovered seven groups or varieties. Each variety has two sex types which unite in conjugation (in one of the varieties at last account only one of the sex types had yet been found). Since the sex types of the different varieties do not conjugate together, but each conjugates with the other sex type of its own variety, there must be at least 13 or 14 diversely reacting sex types in this species, with correspondingly 13 or 14 diverse sex-stuffs.

In Paramecium bursaria five different non-interbreeding groups or varieties have been found. Group or Variety I has four diverse sex types, which have been designated A, B, C and D; Group or Variety II has eight sex types, E, F, G, H, J, K, L and M; Group or Variety III has the four sex types, N, O, P and Q, while Group or Variety IV has but the two sex types, R and S. There are indications that one or two more varieties exist in this species, but these have not been fully studied. Thus in the four well-known groups or varieties of Paramecium bursaria there are 18 diverse sex types, and correlatively there must be 18 diverse sex-stuffs.

Fertile conjugations occur only between individuals of different sex types belonging to the same group or variety. Yet a few cases are known of abortive conjugations between members of different varieties. De Garis in 1935 induced by ingenious methods conjugation between individuals of Paramecium aurelia and Paramecium caudatum, but both members of all pairs died without producing descendants. Sonneborn in 1939 showed that a weak and partial sexual reaction occurs between certain mating types of two different varieties (Variety 1 and Variety 3) of Paramecium aurelia, but conjugation is never completed and no hybrid offspring result. In Paramecium bursaria conjugation may occur between one of the two sex types of Variety IV and any one of four of the eight sex types of Variety II, but the conjugants always die without producing descendants.

In such abortive conjugations between members of different varieties or species, it appears that the sex-stuffs of the two must have an affinity for each other, but the stocks have become so diverse in other respects that successful or fertile conjugation cannot occur.

What is the relation of the, in some instances, numerous "sex types" of Protozoa to the two sexes of higher organisms? The two types that are present in the flagellates have been by Hartmann and Moewus regarded as identical with male and female sexes, and as illustrating the supposed universal differentiation of living material into male and female. The cases in which there are four, six or eight "sex types," as in Paramecium bursaria and Euplotes patella, obviously present difficulties for this point of view. In order not to prejudge the question and while waiting for more evidence to accumulate, the diverse "sex types" of the Protozoa are commonly spoken of by the non-committal term "mating types." Since, however, the differentiations into types in the Protozoa is at least analogous to the differentiation of sexes, it seems appropriate to bring out this analogy by the use of the term "sex types"; hence this term is employed here. For the rest, one must wait for further evidence.

In any case the differentiation into sex types and "varieties" and the change in behavior with change in age result in a social system that is complex. In a population of a given species there occur individuals showing many different kinds of behavior. There are young, immature individuals that do not react sexually; there are adolescents in various grades of development; there are mature individuals. Among any of these may be represented any or all of the varieties and sex types that occur in the species; in Paramecium bursaria, for example any of the eighteen known sex types, distributed into four non-interbreeding varieties. An individual that meets with these various kinds of other individuals varies its behavior in accordance with the place they occupy in the system. If they are of the same sex type as itself, whether young or mature, or if young and immature whatever their variety or type, or if they belong to a different "variety" of the species, there may be no reaction at all; or the two individuals that meet may swim for a time in contact, in coordinated motion, though after a time they separate. If the individual met belongs to the same variety but to a different sex type, there is another kind of behavior; the two adhere together and conjugation occurs. Among the 18 diverse sex types of Paramecium bursaria, any individual of Variety I or Variety III will react sexually with three, but refuse to react with the other 15; an individual of Variety II will react with seven and reject eleven; while, an individual of Variety IV will react fully with but one, partially with four others, and reject completely thirteen.

For more detailed knowledge of the organisms and relations described above the publications mentioned in the appended list may be consulted.

Publications summarizing knowledge on the matters discussed on the foregoing paper:

Sonneborn, T. M., "Sexuality in Unicellular Organisms," Chapter XIV, in Calkins and Summers' *Protozoa in Biological Research,* 1941.

Kimball, R. F., "Mating Types in the Ciliate Protozoa." *Quarterly Review of Biology,* Vol. 18, pages 30-45, 1943.

Jennings, H. S., "The Beginnings of Social Behavior in Unicellular Organisms," *Leidy Memorial Lecture,* University of Pennsylvania Press, 1941.

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97.

STUDIES ON REACTIONS TO STIMULI IN UNICELLULAR ORGANISMS. I. REACTIONS TO CHEMICAL, OSMOTIC AND MECHANICAL STIMULI IN THE CILIATE INFUSORIA.

By HERBERT S. JENNINGS, Ph.D. (Twenty-two Figures in Text.)

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(*From the Physiological Laboratory of the University of Jena.*)

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1. INTRODUCTION.

THE work, an account of which is given in the following pages, consists of a study, as exact as possible, of the life activities of a single unicellular organism. So far as I know, we do not possess an even approximately complete account of such activities as may be referred to reactions to stimuli for any single cell or any single organism. We possess indeed many excellent researches on various forms of reaction in various organisms. But we are not yet able to form a mental picture of the continual interplay of such activities in the ordinary life of any cell; the interaction of the various stimuli; the modifications in reaction due to the simultaneous presence of two or more stimuli; or the changes in reaction due to varying life conditions or physiological states of the organism. Certainly a beginning has been made in unravelling this complicated network of activities, but the studies which have been made are scattered over a wide field;—they are extensive rather than intensive.

Perhaps it may be said that we possess of no unicellular organism so full an account of the life activities as of the common infusorian *Paramœcium aurelia*. The ease with which it may be obtained in great abundance and preserved in healthy condition for indefinite periods has made it a favourite subject for investigation of the life phenomena of free cells. The general anatomy and functions of the parts of this organism are treated fully in the text-books and manuals. The nuclear phenomena in conjugation and division have been made the subject of elaborate studies by distinguished investigators. Turning to more purely physiological matters, the digestive processes of Paramœcium have been studied by Greenwood and Saunders and others; the effects of a lack of oxygen, and of an atmosphere of carbon dioxide by Loeb and Hardesty; the effect of various chemical reagents by Schürmayer, Bokorny and others. The reaction to the electric current has been described by Verworn and by Ludloff: geotaxis by Jensen; thermotaxis by Mendelssohn. Chemotactic and tonotactic movements have been the subject of scattering notices by various authors[1]. The prospect for gaining a fairly full general view of the life activities of this organism is perhaps as favourable as for any that can be named: it was therefore selected for further study.

Of the above-mentioned reactions, not all have been studied in any sense fully. Electrotaxis, geotaxis and thermotaxis may be considered as having been placed on a solid basis by the systematic investigations of Verworn and Ludloff; Jensen, and Mendelssohn. The other reactions mentioned—chemotaxis and tonotaxis—are known only from scattered and partly incorrect notices in the works of various authors. It is the purpose of the present paper to present a systematic account of these and certain other reactions.

In discussing motions which occur as responses to stimuli, two sets of terms are used by different authors, one set having the termination *-taxis* or *-tactic*, the other ending in *-tropism* or *-tropic*. I shall use throughout for the reaction movements of the free infusorian Paramœcium the terminations *-tactic* and *-taxis*, speaking thus of chemotaxis, electrotaxis and the like, instead of chemotropism, electrotropism, etc., often used in the same sense. *Positive* taxis signifies a tendency to move *toward* a source of stimulus; *negative* taxis a tendency to move away from such a source.

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[1] For references see end of Paper.

The reactions to be described are somewhat complicated by the interplay of different stimuli, so that it is difficult to give an account of the phenomena due to any one stimulus without at the same time treating of activities due to different causes. The difficulty in presentation thus caused will perhaps be best avoided by the following procedure. I will first give an account of the phenomena observed under certain simple conditions. The complex of phenomena presented in this "introductory experiment" will then be analysed into its simple components,—that is, into activities due to simple stimuli, and each of these simple reactions will then be treated at length, with details of experiments and other proof of the correctness of the analysis. The Paramœcia used in the experiments were procured in the customary manner. A handful of hay or grass is placed in a jar and covered with hydrant water. In a few weeks the solution of decaying vegetable matter swarms with Paramœcia. In such a jar they may be kept for indefinite periods in almost unlimited numbers.

Introductory Experiment.

The phenomena which we wish to study will be best brought before us by the following simple procedure. A large number of Paramœcia are removed from the culture jar by means of a pipette, and placed, together with a small bit of decaying vegetable material or a mass of bacterial zooglea from the same jar, on a glass slide. The drop of water containing Paramœcia is then covered with a cover-glass resting upon glass rollers or other convenient supports. The preparation thus made is shown in Fig. 1.

At first the Paramœcia are distributed uniformly throughout the preparation, as shown in Fig. 1. In a few minutes however we observe a noticeable gathering of the infusoria about the bit of decaying material. This increases in number and density: the Paramœcia press close against the decaying material, and soon all the infusoria in the preparation (with individual exceptions) are gathered in a dense mass about it. The preparation now presents the appearance shown in Fig. 2. As many as possible of the animals press their anterior ends against the decaying substance: the rest crowd in behind, trying to get as close as possible.

After some minutes we notice that the dense mass of Paramœcia about the decaying material is becoming looser. The Paramœcia begin to separate; they no longer remain closely pressed against the bit of material, but swim hither and thither. However, they do not swim away freely, but remain confined to a small round area in the immediate region of the decaying mass. Very slowly the area becomes larger, but remains clearly defined, with a distinct boundary which the Paramœcia do not pass. Most of the infusoria are gathered at the boundary of the area; some few swim back and forth within it, while scarcely a single specimen will be found outside the area. A swimming individual starting to pass the outer limit stops suddenly, as if prevented by an invisible barrier, and remains or turns back. The limits grow on all sides slowly and steadily, the area thus retaining its regular form. A stage in this process is represented in Fig. 3. The area continues to increase in size, and after some time it reaches the edges of the cover-glass. The Paramœcia then form a narrow band about the periphery of the preparation.

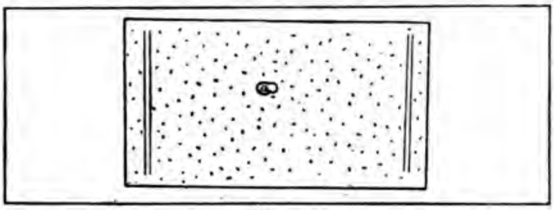


Fig. 1

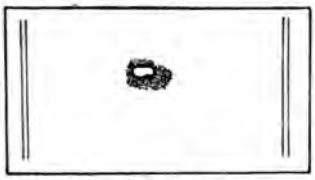


Fig. 2

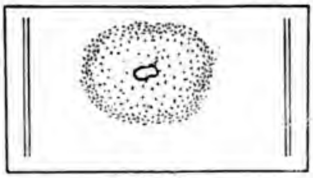


Fig. 3

Fig. 1. Paramœcia which have just been covered with a cover-glass. *a* Bit of decaying vegetable matter.

Fig. 2. Same preparation shown in Fig. 1, ten minutes later.

Fig. 3. The same preparation shown in Figs. 1 and 2, a half hour after the condition shown in Fig. 2.

If the above preparation has been made in such a way that a constant electric current can be passed, by the use of unpolarisable electrodes, through the water containing the Paramœcia, certain very peculiar phenomena may be observed in the reactions of the infusoria to the current. If the electric circuit is closed at the beginning of the experiment, when the Paramœcia are distributed throughout the preparation (Fig. 1), we obtain the typical reaction described by Verworn (1889 (*a*)). The Paramœcia all swim with one accord to the cathode side of the preparation. If the current is reversed the Paramœcia swim to the opposite side. Opening the circuit, the Paramœcia distribute themselves again uniformly throughout the preparation.

If the electric circuit is again closed after the Paramœcia have gathered close about the bit of decaying vegetable material (Fig. 2), no visible effect is produced. The Paramœcia remain gathered about the decaying mass and show no response to the current. My attention was called to this striking phenomenon by Professor Verworn, and it formed the starting point for my investigations.

The circuit may now be opened and the normal development of the preparation observed farther. If the circuit is again closed at the stage shown in Fig. 3, we obtain a still different result. The Paramœcia start in the direction of the cathode, but at that boundary of the circular area which lies next to the cathode they stop. If the current is continued all the Paramœcia gather in a mass at the cathode side of the area, seeming to make vain efforts to cross the invisible boundary. The area has now entirely disappeared, since the Paramœcia are all collected at one spot on its cathode side. If now we reverse the current, the Paramœcia start in the opposite direction, but are again stopped by the invisible barrier, which lies at exactly the spot which previously formed the limit of the area on this side. Opening the circuit, the Paramœcia again distribute themselves in the area, chiefly about its margin, presenting anew exactly the appearance shown in Fig. 3.

What now is the meaning of this peculiar drama? Why do the Paramœcia gather about the bit of decaying vegetable material, placing themselves in contact with its surface? Why do they not respond to the electric current under these conditions? Why do they soon begin to leave the mass, which they first crowded upon with so much ardour? Why do they then remain in a clearly defined growing area, gathered at its boundaries, but not overpassing its invisible limits even when urged by the electric current?

We will consider first the question, Why do the Paramœcia gather about the bit of decaying vegetable material? The conjecture which lies nearest is, that we have here a case of

2. CHEMOTAXIS.

Before entering upon a series of experiments to determine the truth of the above conjecture, let us review briefly what is known of chemotactic phenomena in Paramœcium. **Verworn** (1889, p. 108) and **Loeb und Hardesty** state that Paramœcium is positively chemotactic toward oxygen. **Pfeffer, Verworn**, and **Massart** (1889, p. 558) state that they do not find the Infusoria Ciliata to be positively chemotactic toward other chemical agents. **Loeb und Hardesty** state that Paramœcia are *negatively* chemotactic toward carbon dioxide, and **Loeb und Budget** that they are negative toward acids and alkalies. **Massart** (1889, p. 558) on the other hand says that in accordance with his own researches and those of **Pfeffer**, the Ciliata are "absolument insensible" to chemical substances. He explains their tendency to move away from solutions of chemical substances as due to *tonotaxis*,—that is, it is the result of differences in osmotic pressure, and has nothing to do with the chemical quality of the solutions. We might infer from the above summary that the only facts regarding chemotaxis that can be stated with any degree of certainty are (1) that Paramœcium is positively chemotactic toward oxygen; (2) that it is probably negatively chemotactic toward CO_2.

It now remains to test the matter by experiment. For this purpose results were obtained by two methods. The first is **Pfeffer's** method of the introduction of capillary tubes containing the substance in question beneath the cover-glass of a preparation, or into a vessel, containing Paramœcia. If the animals are positively chemotactic to the substance in the tube, they gather into its open end. Many experiments were tried in this way, but a second method was found to give results incomparably quicker, more definite, and more reliable. The Paramœcia are placed on a glass slide and covered by a supported cover-glass as described in the "Introductory Experiment." A pipette is drawn out at the end into a capillary tube of sufficient fineness to pass between the slide and cover into the water containing the Paramœcia. The latter swim at first hither and thither in every direction, so that no portion of the preparation is left free. Now a drop of the fluid to be tested is taken up with the capillary pipette and introduced beneath the cover-glass into the preparation (Fig. 4). The drop fills the space between slide and cover, and spreads out into a round disk, so that it is in contact only by the very thin edge of the disk with the surrounding water; it therefore diffuses only very slowly, as may be seen by introducing a drop of some coloured fluid. The Paramœcia in their random hither and thither swimming of course begin at once to come in contact with the edge of the drop, whereupon their conduct toward the substance of which the drop is composed may be observed. When I speak later of a drop of fluid being introduced beneath the cover-glass it will be understood that it was done in the way just described.

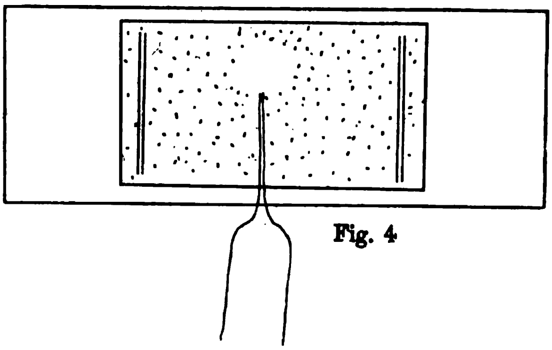


Fig. 4

Method of testing the behaviour of Paramœcium toward chemical substances.

The first question to be tested is, Are the Paramœcia positively chemotactic toward the decaying vegetable material of the culture jar? Since Paramœcia taken from the culture jar are already bathed on all sides by fluid from this decaying vegetable material, they cannot be used at once for a decisive test of the question; before a test can be made they must be brought into water containing none of this fluid. To accomplish this, I have used a method similar to one described by Ludloff (p. 529) as a convenient way of bringing many Paramœcia into a small amount of water. A glass tube 1½ cm. in diameter and 80 cm. in length, closed at one end, was filled about half full of fluid from the culture jar, containing large numbers of Paramœcia. The remaining half of the tube was filled with hydrant water, and the tube allowed to stand in an upright position. The Paramœcia, being negatively geotactic, rise to the upper end of the tube into the hydrant water. They are then poured into another vessel, the tube emptied and thoroughly cleaned, and the Paramœcia restored to the lower half of the tube. Hydrant water is again added in the upper half and the Paramœcia rise into it as before. This may be repeated as often as thought necessary, till the Paramœcia are washed entirely free from the fluid of the culture jar.

A quantity of this pure hydrant water containing Paramœcia is then placed on the slide and covered in the ordinary way. The Paramœcia scatter uniformly throughout the preparation. Some fluid is now drained from the decaying vegetable material of the culture jar and filtered. A drop of this filtered fluid is then introduced beneath the centre of the cover-glass. The Paramœcia begin at once to gather in the drop. In a few minutes there is a marked collection of individuals in this region, and after a short time all the Paramœcia in the preparation, with individual exceptions, are gathered in the small circular area which marks the spot where the drop was introduced (Fig. 5), the rest of the slide being quite empty.

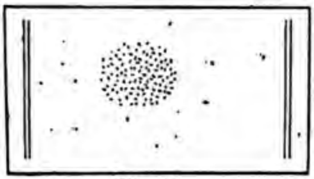


Fig. 5

Similar results were gained by introducing capillary tubes containing filtered fluid drained from the decaying vegetable material of the jar into vessels of hydrant water containing many Paramœcia. A typical experiment of this sort is as follows. Paramœcia in hydrant water are placed in a shallow watch-glass. Into this glass are then introduced pieces of capillary tubing, some containing filtered fluid from the culture jar, the others hydrant water. These are arranged in pairs, each pair consisting of two tubes of the same length and diameter, lying side by side, one containing the fluid from the jar, the other hydrant water. In about three-quarters of an hour the ends of the tubes containing fluid from the culture jar are crowded with Paramœcia, while the others are empty.

Paramœcium aurelia in hydrant water thus tends to gather into areas or tubes containing water from a culture jar of decaying vegetable material. Apparently the infusoria are positively chemotactic toward such material. In order to avoid misconception, I may state at once that this conclusion is shown by the remainder of the investigation to be false, and the results gained by the above experiments are to be explained in an entirely different manner, as will be set forth in due time.

Experiments were now tried with many other substances, using both the above methods. The latter method—introduction of capillary tubes into a vessel containing many Paramœcia—is not at all reliable, so that I gradually ceased to use this, and my results depend almost entirely upon experiments with the other method. Certain reasons why the capillary tube method is unreliable will be given later.

With Paramœcia in hydrant water a filtered extract of meat was tested. This was prepared by boiling a bit of lean beef in water and filtering off the fluid. The Paramœcia gather into a drop of this exactly as into a drop of the fluid from the culture jar. Meat extract prepared by soaking lean meat in cold water acts in the same way, as does also Liebig's meat extract.

The infusoria were also positive toward *rancid oils*, such as rancid olive-oil and rancid cod-liver oil. Paramœcia gather quickly into a drop of water which has been well shaken up with such oils and introduced beneath the cover-glass of the Paramœcium preparation.

With many other substances no such gathering occurred. Cane sugar, salt, a pepsin solution, putrid meat juice, putrefying gelatine, saliva, egg albumen, all gave negative results: the Paramœcia were either indifferent or repelled by these substances.

The same results were gained with all the above substances if the Paramœcia tested were left in the water from the culture jar instead of being brought into hydrant water.

The positive results gained with rancid oils suggested that the active attractive principle was some member of the fatty acid series. I determined therefore to test various members of this series. During the short delay necessary for getting samples of the acids desired, I directed my attention to certain other phenomena, the study of which placed the whole problem in a new light. As previously stated, Massart (1889, p. 558) maintains that the apparently negative chemotaxis of the ciliate infusoria is not really chemotaxis at all, but is due merely to differences in osmotic pressure between the fluid the animals are already in and that of the fluid introduced; the motions due to this stimulus he calls tonotaxis. Desiring to test this matter for Paramœcium, in the case of substances apparently negatively chemotactic, I undertook first to determine the weakest concentration to which the Paramœcia are negative, in the case of some strong chemical. For this purpose *copper sulphate* was selected: the saturated solution was diluted until experiment proved that it was not quickly fatal to Paramœcia introduced directly into it, and tests were made with this. The strength thus used was $\frac{1}{80}$ % of the saturated solution; a drop of this was introduced beneath the cover-glass of a Paramœcium preparation (cp. Fig. 4).

The Paramœcia were, contrary to all expectation, distinctly positively tactic to the copper sulphate. They formed a dense collection about the margin of the drop, but did not enter into its centre, being apparently negatively tactic to the stronger solution within. The infusoria seemed not to be injured at all by the fluid forming the margin of the drop, but were much excited, swimming hither and thither with great rapidity, but not leaving the drop of copper sulphate.

In view of this surprising result with copper sulphate a number of other strong chemicals were tested, some with positive, others with negative results. Without going at this point into details, I will give here a table showing in general the results gained with the chemicals tested. Positive taxis is indicated by the sign (+), negative taxis by the sign (–). The Paramœcia were tested in the fluid from the culture jar. Solutions of the chemicals made up both with distilled water and with water from the culture jar were tried: the same results were gained by both methods.

Substance	Weak sol.	Strong sol.	Substance	Weak sol.	Strong sol.
Copper sulphate	+	–	Sodium chloride	–	–
Sulphuric acid	+	–	Sodium carbonate	–	–
Hydrochloric acid	+	–	Sodium bicarbonate	–	–
Acetic acid	+	–	Potassium hydroxide	–	–
Nitric acid	+	–	Sodium hydroxide	–	–
Tannic acid	+	–	Potassium bromate	–	–
Mercuric chloride	+	–			

From the table it appears (1) that all acids tested were positive (that is, attractive) in a weak solution; negative in a strong solution: (2) that all alkalies tested were negative in whatever strength used: (3) that some salts act positively, others negatively. Those salts which are attractive in a weak solution give an acid reaction ($CuSO_4$ and $HgCl_2$); salts giving an alkaline reaction are negative, as are also certain neutral salts.

I now undertook to determine the *weakest* solution of certain of these chemicals, to which the Paramœcia show sensibility. The first substance thus tested was sulphuric acid. The concentrated solution was mixed with distilled water and the solutions thus gained were used in testing the Paramœcia, the latter being in water from the culture jar.

The Paramœcia seemed to show a perfectly astounding sensitiveness to the dilute H_2SO_4. Solutions of $\frac{1}{1000}$ %, $\frac{1}{2000}$ %, $\frac{1}{4000}$ %, $\frac{1}{8000}$ %, $\frac{1}{16000}$ %, were successively used; in every case the Paramœcia showed strong positive taxis, gathering quickly into the introduced drop. Finally, as a control, a drop of pure distilled water was introduced in the ordinary way. The Paramœcia showed strong positive taxis to the distilled water. They gathered into the drop, forming a dense collection, exactly as shown in Fig. 5.

This result set the entire problem in a new light, and compelled a re-interpretation of all previous observations. To say that the Paramœcia are positively chemotactic toward distilled water is meaningless; we must reverse the statement and say that Paramœcia are negatively chemotactic toward the fluid of the culture jar in which they live.

We have seen above that the infusoria are negative to all alkalies and positive to acids. The fluid of the culture jar in which the Paramœcia live shows with litmus paper a distinctly alkaline reaction. Thus however remarkable it may seem that Paramœcium should be negatively chemotactic to the fluid in which it lives and thrives, the reaction stands in entire agreement with the behaviour of the animal toward other reagents.

The conclusion is further reinforced by the exact method in which the Paramœcia react to the various substances tested. Certain features are common to all the experiments. Suppose a slide and cover preparation has been made, in which the Paramœcia are distributed uniformly, and a drop of some substance is introduced as in Fig. 4. The behaviour of the Paramœcia at even a slight distance only from the drop is not thereby influenced in the least: the drop has no effect at an appreciable distance from its evident margin. But all the Paramœcia continue to swim hither and thither at random, and in a remarkably short time almost every individual under the cover-glass will have come in contact with some part of the drop. There is however no swimming in straight radial lines to the drop as a centre. It is only as the Paramœcia come by chance in contact with the drop that their conduct toward the substance of which it is composed can be observed.

With this preliminary general statement, we may now study the conduct of the Paramœcia on coming in contact with (1) a drop of distilled water, (2) a drop of weak acid, (3) a drop of alkali. The Paramœcia in every case are in the natural water from the culture jar, having an alkaline reaction.

Suppose therefore that a drop of distilled water is introduced. A Paramœcium in its random swimming comes against the margin of the drop. Just what happens here varies with individuals; some make a sudden pause, often jerking back a fraction of their own length, then passing into the drop; others show not the slightest hesitation of any sort at the boundary. In any case the animal continues its course with undiminished rapidity until it comes to the opposite boundary of the drop, where it would naturally pass out into the culture fluid again. Here it leaps back perceptibly,—the effective stroke of the cilia being reversed in direction,—turns, and swims in another direction. Again meeting the boundary it draws back, turns again, and swims till it again strikes the boundary. It thus continues to swim about in the drop as if caught in a trap, the changes of motion indicating always negative chemotaxis to the surrounding fluid. Fig. 6 represents the course of a single Paramœcium entering a drop of distilled water and remaining as above described. In a short time the drop thus becomes crowded with individuals, and the preparation shows exactly the appearance represented in Fig. 5.

If in place of distilled water we introduce a drop of $\frac{1}{100}$ % H_2SO_4 the conduct of the Paramœcia is somewhat different. On swimming by chance against the edge of the drop the animal suddenly reverses its cilia and swims a long distance straight backward, often ten times its own length, or much more. It then either starts off in another direction, in which case of course it leaves the drop entirely, or it swims straight forward again to the margin of the drop. Here the reversal of the cilia occurs again, but the animal generally does not swim so far backward as before. This may be repeated a number of times, until finally the Paramœcium does not reverse its cilia and swim backward on striking the drop, but merely turns away and swims in another direction. Here however it comes to another invisible boundary at a slight distance outside of the evident margin of the drop of acid, from which it turns again toward the interior,—turns from this as at first only to meet the outer boundary again, and thus it continues to swim in a ring about the drop of acid, repelled by both the inner and

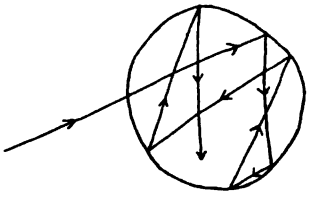


Fig. 6

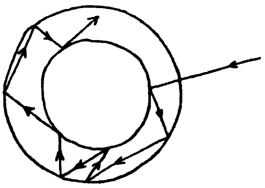


Fig. 7

Fig. 6. Course of a Paramœcium in a drop of distilled water.
Fig. 7. Course of a Paramœcium about a drop of $\frac{1}{100}$ % sulphuric acid.

outer boundary of the ring. The course of a Paramœcium in such a case is shown in Fig. 7. As the surrounding medium is alkaline while the drop itself is acid, it appears that the ring to which the Paramœcia are confined must be nearly or quite neutral in reaction. The outer boundary indicates negative chemotaxis toward alkaline fluid: the inner boundary indicates negative chemotaxis toward (strong) acid. Such a ring becomes in a few minutes densely crowded with Paramœcia; Fig. 8 shows a preparation into which was introduced five minutes before a drop of $\frac{1}{100}$ % H_2SO_4. If a very weak solution of H_2SO_4 is used —about $\frac{1}{800}$ %—the Paramœcia act toward it exactly as toward distilled water. If a very strong solution of acid is used, the results above described are modified in one respect. Many of the Paramœcia, swimming too violently against the inner boundary of the ring are there killed by the strong acid before they can swim away, so that the inner boundary is indicated by a zone of dead Paramœcia.

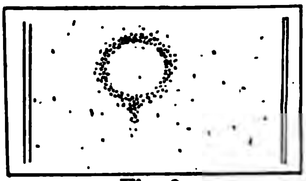


Fig. 8

Paramœcia gathered in a close ring about a drop of $\frac{1}{100}$ % H_2SO_4.

Essentially similar results are gained by the use of proper degrees of concentration of any of the other acids mentioned, or with the acid salts, copper sulphate or mercuric chloride.

If after the Paramœcia have gathered in the drop of weak acid or of distilled water the constant electric current is passed through the preparation, the same results are gained as described on p. 262, for the group formed in the "introductory experiment." The Paramœcia swim to the cathode side *of the drop*, but no further; nor can they be forced over the boundaries of the drop without a very strong and long-continued current. If the substance is a drop of stronger acid, so that the Paramœcia are confined to a ring around the outside of it, being negative to the centre (Fig. 8), when the electric circuit is closed the Paramœcia do not swim across the drop, but follow around its circumference, from one side to the other.

Finally, if we introduce into a preparation a drop of some alkali —for example, a $\frac{1}{200}$ % solution of KOH, in place of the distilled water or the acid, an entirely different result is gained. The Paramœcia on coming in contact with the margin of the drop, reverse their cilia, though much less violently than in the case of a strong acid, and jerk back, perhaps a fraction of their own length. At the same time they turn and swim in another direction. The drop thus remains entirely empty: there is no collection either within it or at its margins. The Paramœcia are not injured by it as they are by an equally powerful acid, because they form no gathering about its margin and therefore do not venture too deeply into it for safety.

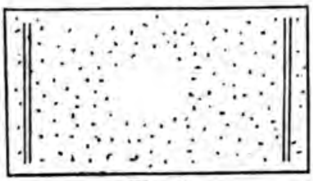


Fig. 9

Preparation into which was introduced 10 minutes before, a drop of $\frac{1}{120}$ % Na_2CO_3.

The same results are gained by the use of other alkalies or salts having an alkaline reaction. Fig. 9 shows the effect of the introduction, 10 minutes before, of a $\frac{1}{120}$ % solution of Na_2CO_3.

In hydrant water the results are the same and possibly even more pronounced than in the water from the culture jar, as the Paramœcia are even more strongly negative to the hydrant water. This is demonstrated by testing Paramœcia in culture water with hydrant water; they are negatively chemotactic to it. The converse experiment—the introduction of a drop of water from the culture-jar into a preparation of Paramœcia in hydrant water—gives the opposite results; the Paramœcia in hydrant water are positively chemotactic to the fluid from the culture jar. The hydrant water in Jena is strongly impregnated with calcium carbonate, and gives a distinctly alkaline reaction with litmus paper, so that negative chemotaxis toward it is what might be expected.

I must mention here a peculiar variation in the results gained, which at first caused confusion in my own observations, and would doubtless do the same in the case of anyone repeating these experiments. The Paramœcia in the culture jars are not distributed uniformly in the water, but are gathered in dense collections,—on the decaying vegetable matter just below the surface of the water, as well as forming a broad white line on the walls of the vessel a few millimeters below the water surface. If for making the tests Paramœcia are taken by means of a pipette directly from one of these collections, and brought in the same pipette under the cover-glass, results entirely different from those above detailed will be gained on the introduction of a drop of the various chemical reagents. The Paramœcia show themselves under these circumstances distinctly and strongly *negative* to every substance introduced, including distilled water and acids. If however the Paramœcia on being removed from the culture jar, are brought first into a watch-glass, and are there thoroughly stirred up and brought into contact with the air, by the use of the pipette, and the tests are then made with slide and cover preparations of these Paramœcia, the results gained are those above described. The negative results gained when the Paramœcia are not thus stirred up are due to the presence in the water of a volatile substance to which they are much more strongly positive than to any of the solutions introduced. This is shown by their beginning at once to gather into groups in different parts of the preparation, avoiding throughout the introduced drop. To determine what this volatile attractive substance is, is one of the chief objects of this portion of the paper.

We may summarize our results up to this point as follows: The Paramœcia live in a fluid having an alkaline reaction. They are negatively chemotactic to this fluid and to all other alkaline fluids. As a result of this negative chemotaxis, they gather into a drop of distilled water introduced into the fluid in which they live. The Paramœcia are also negative to strong acids, but gather quickly into a weakly acid fluid introduced into the alkaline water in which they live. Whether this is because the Paramœcia are really positively chemotactic to the acids or only gather in a region where the acid neutralizes the alkali of the surrounding fluid is not yet determined.

The next question for determination is therefore whether the Paramœcia are really positively chemotactic toward acids, or whether all the phenomena observed can be explained by the negative chemotaxis toward alkalies. In order to test this question the Paramœcia must first be brought into an entirely neutral medium,—that is, into distilled water.

The infusoria are easily brought into distilled water by the use of the glass tube, taking advantage of the negative geotaxis of the animals, as described on p. 264; distilled water is simply substituted for hydrant water in the procedure there described. The Paramœcia are not appreciably harmed by the transfer and live in the distilled water for weeks, seemingly as lively as ever. In the first one or two days after the transfer is made the animals are excessively sensitive to all chemicals, showing marked negative chemotaxis to almost all substances tested, and positive chemotaxis to almost none. But after three or four days or a week in distilled water the conduct becomes quite normal, except that they are much more sensitive to chemical agents than under normal circumstances.

Slide and cover preparations were made as before, with the Paramœcia in distilled water, and the same substances were re-tested under these circumstances. This is a piece of work requiring great pains and much time. The sensitiveness of the Paramœcia to chemical reagents is so increased that solutions of acids which formerly acted positively now have a distinct negative effect. In order to be certain whether positive chemotaxis toward a given substance occurs at all or not, many experiments are necessary to discover the precise degree of concentration which acts positively. This is further complicated by a peculiar fact. After the Paramœcia have remained some days in distilled water, they become positively tactic to distilled water in which Paramœcia have *not* lived. The explanation of this fact is probably that the distilled water containing many Paramœcia becomes slightly contaminated by matter excreted by the animals. I have tested such water carefully with litmus paper, but was unable to discover any evidence of its having gained an alkaline reaction. However, the fact renders it inadmissible to mix the reagents with ordinary distilled water, since of course if the concentration of the substance to be tested is lowered to such an extent that it becomes entirely ineffective, the *positive* reaction due to the simple distilled water is gained, leading to false conclusions.

It was therefore necessary to mix the reagents with the *same* water in which the Paramœcia to be tested are swimming. The only certain method is to have the Paramœcia, in distilled water, in a vessel of some size; then to remove from this same vessel with a pipette water (containing Paramœcia, of course) for mixing with the reagents. It is even necessary to keep the vessel thoroughly stirred up, as otherwise the Paramœcia in one part of it may become positively or negatively tactic toward water from a different part of the same dish,—a peculiar fact of which the explanation will be given later.

However, all these precautions being observed, the following results are gained. Toward all the substances given in the list on page 283 to which the Paramœcia in the water from the culture jar were negatively chemotactic, the Paramœcia in distilled water are likewise negatively chemotactic. In the latter case the negative chemotaxis is more powerful, inasmuch as solutions which are so diluted as to be entirely ineffective in the former case, are strongly negative in effect if the Paramœcia are in distilled water.

Toward substances with regard to which the Paramœcia in the water from the culture jar are positive, the Paramœcia in distilled water are likewise positive. But in the latter case the positive chemotaxis is much *weaker* than with Paramœcia in water from the culture jar. The positive chemotaxis occurs, when the Paramœcia are in distilled water, only with very weak solutions, and all stronger solutions of the same substance act negatively. Moreover, the collections of Paramœcia formed are scarcely so dense and often do not last so long as in the previous experiments.

The greatly increased sensitiveness in distilled water, indicated by the changed chemotaxis, is shown also in another way. Chemical solutions which had almost no injurious effect on Paramœcia under natural conditions, are quickly fatal to Paramœcia in distilled water.

We may conclude from the foregoing series of experiments that Paramœcium is, (1) positively chemotactic toward substances having a weak acid reaction; (2) negatively chemotactic toward alkalies and some salts. The results given in the beginning (page 266) for certain complex substances readily take their place under this view. Rancid oils have of course an acid reaction, and the same is true of extract of meat; hence the positive chemotaxis toward these substances. The apparently positive chemotaxis of Paramœcia in hydrant water to fluid from the decaying vegetable material in the culture jar is seen to be only an expression of the fact that they are less strongly negative to the water of the culture jar than to the hydrant water. Paramœcia in distilled water are strongly negative both to water from the culture jar and to hydrant water, these both having an alkaline reaction.

Relation of Chemotaxis to Tonotaxis, and the actual rôle played by Tonotaxis.

There remains undecided, however, the relation of the phenomena described to Massart's tonotaxis. Probably no one would invoke the aid of tonotaxis to explain what I have described above as *positive* chemotaxis. But is not what I have heretofore spoken of as negative chemotaxis perhaps of the nature of Massart's tonotaxis? Tonotaxis signifies a motion of free organisms as a reaction to a stimulus due to a change in the osmotic pressure of the surrounding fluid.

It supposes that when an organism is living normally in a fluid of a certain osmotic pressure an increase in this osmotic pressure will cause fluid to pass out of the organism, a decrease in this osmotic pressure will cause fluid to pass into the organism, and that the passage of fluid in either case is a stimulus to the organism causing it to move.

The question, therefore, which we wish to decide is, whether the negative taxis of Paramœcium in coming into contact with solutions of various substances is due to the osmotic pressures of the solutions or to their chemical characters. Obviously the way of testing this is to determine how far the reactions of the Paramœcia vary with variations in the osmotic pressure, and how far they vary with variations in the chemical nature of the surrounding fluid.

In working this out the method I have followed is to find the weakest solution of any substance *A*, to which the Paramœcia show a recognizable negative taxis, to determine the same point for a second substance *B*, and then to compare the osmotic pressures of the two solutions. If the two solutions have the same osmotic pressures, *i.e.* are isotonic, the evidence is in favour of the theory that the taxis is the result of the osmotic pressure, otherwise not.

In estimating the percentage of a substance *B* which is isotonic to a given percentage of a substance *A*, I make use of the formula

$$\text{p.c. of } A\ \frac{(\text{molecular weight of } B)\ (i \text{ of } A)}{(\text{molecular weight of } A)\ (i \text{ of } B)} = \text{p.c. of } B,$$

and of the molecular weights and values for *i* given in the following Table. The values for *i* I have taken from the works of de Vries, Hamburger, Köppe and Arrhenius. The variations from the true value which there may be in some instances are too slight to affect the results obtained in this investigation.

TABLE I. *Substances tested for Tonotaxis.*

Substance	Mol. wt.	Value of *i*.	Substance	Mol. wt.	Value of *i*.
Potassium hydroxide	56	1·92	Ethyl alcohol	46	1·00
Sodium hydroxide	40	1·92	Chloral hydrate	147·5	1·09
Sulphuric acid	98	2·12	Cane sugar	342	1·00
Hydrochloric acid	36·5	1·94	Dextrose	180	1·00
Sodium chloride	58·5	1·66	Mannite	182	1·00
Sodium carbonate	106	2·20	Glycerine	92	1·00
Potassium bromate	167	[1·61]	Urea	60	1·00
Copper sulphate	249	1·16			

The experiments were carried on by means of the slide and cover preparations and introduced drop (cp. Fig. 4). The solutions were made by dissolving the proper proportions of the substances by weight in distilled water: thus a 2 °/₀ solution would signify a solution made by adding 2 g. of the substance to be tested to 98 cc. of water. Throughout the experiments I have used a solution of ordinary salt (Na Cl) as a standard with which to compare other substances. This substance was chosen as a standard for several reasons. It has a decidedly negative action wherever present in effective quantities. But this negative action is not violent, so that the solutions become ineffective without being excessively diluted; this seemed to indicate that the action might be purely osmotic in nature, making it a favourable substance for comparison with others. Of course this adoption of a standard is purely a matter of convenience; the general results will be the same whatever the standard used.

The experiments are much more difficult in the execution, if accurate results are to be obtained, than might appear at first. The plan is as follows. Paramœcia in the water of the culture jar or in hydrant water are positively tactic toward distilled water, so that if a drop of distilled water is introduced with the capillary pipette, it is quickly filled with Paramœcia. If, however, some substance to which the Paramœcia are negatively tactic is dissolved in the distilled water the drop remains empty on being introduced into the preparation. Exactly that concentration must now be sought out to which the Paramœcia are nearly or quite indifferent: that is, such a concentration that they enter the drop on coming in contact with it, but do not form a collection within it. If a higher concentration is used they will not enter the drop, while if a lower concentration is employed they act toward it as toward a drop of distilled water. This indifference point can be determined with a close approximation to accuracy. But it is not possible to determine this point for any standard substance, as, say, Na Cl, once for all, because of the great variations in reaction under varying circumstances. Among the many circumstances to be taken into consideration are the following. First, the Paramœcia in the culture fluid are not always positive toward distilled water, so that this point must be carefully tested beforehand. Of course if the experiments are carried on with Paramœcia that are negative to distilled water it is impossible to determine the point where the negative action of the substance in solution ceases. Secondly, having tested the Paramœcia and found them positive to distilled water—they may soon become negative to it if they are not prevented by occasionally stirring them up thoroughly. Thus the later members of a series of experiments will show entirely different results from the first ones, and the entire series is rendered worthless through a neglect of this precaution. Thirdly, Paramœcia at different times and under different circumstances show an entirely different degree of sensibility to the same solution. These remarkable variations will all find a satisfactory explanation at a later point in the investigation. Fourthly, the reaction depends to a certain extent on the thickness of the layer of water between the slide and cover-glass. If the space between the slide and cover is great, the introduced solution diffuses more quickly and the reaction of the Paramœcia is therefore not so pronounced. Hence the results of experiments carried on with the cover-glass resting on supports of varying thickness are not comparable.

All these precautions are satisfied by the following method of procedure. Paramœcia are removed from the culture jar with a large pipette, placed in a small beaker and thoroughly stirred up and brought into contact with the air. Thereupon, as before stated, they become positive to distilled water. This stirring must be repeated often to prevent their becoming again negative. A preparation is then made by bringing the Paramœcia upon a slide with a long cover-glass (22 mm. by 40 mm.), supported near its ends by two pieces of capillary tubing of equal and uniform diameter. A number of beakers are arranged, one containing a solution of $NaCl$ of about the strength that will probably (from previous trials) be found to be nearly neutral; the second a solution, isotonic with the $NaCl$ solution, of the other substance to be tested; a third containing distilled water. A drop of the $NaCl$ solution is taken up with the capillary pipette and introduced beneath the cover-glass, but near one end, forming there a small circle 5—8 mm. in diameter. A drop of the second solution to be tested is introduced in the same manner near the *other* end. The two drops are thus under precisely similar conditions, and the conduct of the Paramœcia toward them can be observed without fear of disturbance of the results by varying outer conditions. The tests must however be made *at once* after the Paramœcia are brought on to the slide, otherwise clear results will not be gained.

It will be understood from the above description of the method of experimentation, that if in one case the lower limit to which the animals are sensitive is given for an $NaCl$ solution as 0·10 %, in another as 0·033 %, there is no contradiction involved, and the conclusions to be drawn are not thereby influenced, since the other substance for comparison was tested in each case under exactly the same conditions as the $NaCl$.

Where substances to which the Paramœcia are *positively* chemotactic (to a weak solution) are found in the table (acids), it is of course to be understood that we are dealing here with the weakest solution to which the Paramœcia are *negative*: to a still weaker solution they are positive.

The results for the different substances are given in Table II. In this table the fluid in which the Paramœcia were tested is given, that is, whether the Paramœcia in this experiment were in water from the culture jar, hydrant water, or distilled water. This should of course make no difference to the results gained, as the two substances (NaCl for comparison and the substance under discussion) were tested under the same conditions. The concentration of NaCl to which the Paramœcia are in this case exactly indifferent is stated, and the concentration of an *isotonic* solution of the substance in question; that is, a solution which has the same osmotic pressure as the above NaCl solution. If the cause of the negative taxis of the Paramœcia lies in the osmotic pressure, we ought to find experimentally also that this is the weakest solution to which the Paramœcia are negative. The minimum to which the Paramœcia are negative, as actually found by experiment, is then given, and this is followed by a statement of the ratio between the *calculated* concentration and this actual concentration observed. To be favourable to the tonotaxis theory, this ratio should of course be 1 or in the neighbourhood of 1.

TABLE II.

Bases.

1. Potassium hydroxide. Paramœcia in hydrant water.
   Indifferent to 0·10 % NaCl, isotonic with 0·083 % KOH.
   Actual minimum observed for KOH, 0·005 %.
   Ratio of calculated minimum to actual minimum, 16 to 1.
2. Sodium hydroxide. Paramœcia in culture water.
   Indifferent to 0·066 % NaCl, isotonic with 0·039 % NaOH.
   Actual minimum, 0·0025 % NaOH. Ratio, 16 to 1.

Acids.

3. Sulphuric acid. Paramœcia in culture water.
   Indifferent to 0·05 % NaCl, isotonic with 0·065 % H_2SO_4.
   Actual minimum, 0·00125 % H_2SO_4. Ratio 52 to 1.
4. Sulphuric acid. Paramœcia in distilled water.
   Indifferent to 0·033 % NaCl, isotonic with 0·042 % H_2SO_4.
   Actual minimum, 0·00125 %. Ratio 33 to 1.
5. Hydrochloric acid. Paramœcia in culture water.
   Indifferent to 0·05 % NaCl, isotonic with 0·026 HCl.
   Actual minimum, 0·00125 %. Ratio 20 to 1.

Salts.

6. Sodium carbonate. Paramœcia in hydrant water.
Indifferent to 0·066 $^{\circ}/_{0}$ NaCl, isotonic with 0·090 $^{\circ}/_{0}$ Na_2CO_3.
Actual minimum, 0·004 $^{\circ}/_{0}$. Ratio 22 to 1.

7. Sodium carbonate. Paramœcia in distilled water.
Indifferent to 0·033 $^{\circ}/_{0}$ NaCl, isotonic with 0·045 $^{\circ}/_{0}$ Na_2CO_3.
Actual minimum, 0·00208 $^{\circ}/_{0}$. Ratio 22 to 1.

8. Potassium bromate. Paramœcia in distilled water.
Indifferent to 0·066 $^{\circ}/_{0}$ NaCl, isotonic with 0·19 $^{\circ}/_{0}$ $KBrO_3$.
Actual minimum, 0·0208 $^{\circ}/_{0}$. Ratio 9 to 1.

9. Copper sulphate ($CuSO_4 + 5H_2O$). Paramœcia in culture water.
Indifferent to 0·05 $^{\circ}/_{0}$ NaCl, isotonic with 0·304 $^{\circ}/_{0}$ $CuSO_4$.
Actual minimum, 0·00125 $^{\circ}/_{0}$ $CuSO_4$. Ratio 243 to 1.

Organic Compounds.

10. Ethyl alcohol. Paramœcia in culture water.
Indifferent to 0·10 $^{\circ}/_{0}$ NaCl, isotonic with 0·13 $^{\circ}/_{0}$ alcohol.
Actual minimum, 1 $^{\circ}/_{0}$ alcohol. Ratio 1 to 8.

11. Chloral hydrate. Paramœcia in culture water.
Indifferent to 0·05 $^{\circ}/_{0}$ NaCl, isotonic with 0·19 $^{\circ}/_{0}$ CCl_3COH.
Actual minimum 0·066 $^{\circ}/_{0}$ CCl_3COH. Ratio 3 to 1.

From this table it is evident that the negative taxis of Paramœcium to these substances bears no relation to the osmotic pressure of the solutions. Taking NaCl as unity, the ratio of the strength of solution reckoned on the basis that the osmotic pressure is the active agent in the stimulus, to the strength of solution actually found varies all the way from 243 to $\frac{1}{8}$. There can be no question therefore that we are dealing here with negative chemotaxis and not with tonotaxis. A considerable number of other substances were tested with sufficient precision to determine that their negative taxis was much stronger than could be reckoned on the tonotaxis theory,—though not with such exactness that they could be taken into the above table.

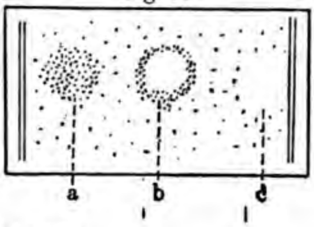
The conclusion that we are dealing with negative chemotaxis and not with tonotaxis is rendered exceedingly probable by certain other considerations. The Paramœcia live in water which contains a considerable quantity of various salts, especially $CaCO_3$, so that a solution in distilled water of, for example, $\frac{1}{800}$ $^{\circ}/_{0}$ copper sulphate is probably much less dense than the water the Paramœcia are already in. The disinclination of the Paramœcia to enter such a solution might possibly be considered negative tonotaxis to a less dense solution than that to which the animals are accustomed; tonotaxis of this sort is not uncommon, according to Massart. But this view is of course rendered inadmissible by the fact that the Paramœcia *do* enter the distilled water, which is still less dense than the $\frac{1}{800}$ % $CuSO_4$. I have thought it best however to make a thorough test of the matter from the other point of view, in order to set the question entirely at rest. Whether we consider the solutions used as denser or less dense than the water in which the Paramœcia are found, it is equally impossible to hold the reactions to be due to tonotaxis.

But the impossibility of considering the above reactions to be due to the osmotic pressure of the solutions is rendered, not more certain, but more striking, when certain other substances are drawn into the circle of experimentation. By the aid of these we are able to see precisely the relations of chemotaxis to tonotaxis, and to observe the effects of tonotaxis acting alone. Toward a number of organic compounds—cane sugar, dextrose, mannite, glycerine, and urea—the Paramœcia are chemically entirely indifferent, so that with them tonotaxis may be studied in its simplicity. This will be best shown by detailing certain experiments.

(1) An ordinary slide and cover preparation is made, with Paramœcia in water from the culture jar. They are tested and found to be positive to distilled water. Now with the capillary pipette a drop of a 2 % solution of cane sugar in distilled water is introduced. The Paramœcia act toward it exactly as toward a drop of distilled water. On coming to the edge they either hesitate an instant, or cross at once, swimming *into* the drop, but stopping on coming to the opposite side, exactly as described on p. 268 for distilled water. Thus in a short time most of the Paramœcia in the preparation are gathered into the drop of 2 % sugar solution.

A 2 % solution of cane sugar has the same osmotic pressure as a $\frac{1}{8}$ % solution of NaCl. Introducing a drop of $\frac{1}{8}$ % NaCl in distilled water into the preparation, the Paramœcia are strongly negative. Successive trials are made with weaker solutions of NaCl, until it is found that the Paramœcia become barely indifferent to a $\frac{1}{20}$ % solution of NaCl. This therefore works more powerfully than a solution of sugar having four times its osmotic pressure. The same Paramœcia were tested with $CuSO_4$, and it was found that while the margins of the drop exercise a positive attraction to the Paramœcia, they are negative to the inside of a drop of $\frac{1}{800}$ % $CuSO_4$ in distilled water. They are thus more strongly negative to a solution of copper sulphate than to a solution of cane sugar having about 1000 times the same osmotic pressure. Fig. 10 shows a preparation to which a drop of a 2 % cane sugar solution at *a*, a drop of $\frac{1}{800}$ % $CuSO_4$ at *b*, and a drop of $\frac{1}{20}$ % NaCl at *c* had been added five minutes earlier.

Fig. 10



The apparently positive taxis to the sugar solution is due merely to the fact that the solution is made up in distilled water, as is shown by the following experiments:

(2) Paramœcia in distilled water. Introduced a drop of 2 % sugar solution in distilled water. The Paramœcia are entirely indifferent to it; they swim in or out of the drop, exactly as in any other part of the preparation.

(3) Paramœcia in water from the culture jar. A 2 % solution of cane sugar was made up also in the culture water, and a drop of this introduced into the preparation. The Paramœcia are entirely indifferent, as in the previous experiment.

(4) Paramœcia in culture water. Under one end of the cover-glass a drop of 2 % sugar solution in distilled water is added; under the other a drop of pure distilled water. The Paramœcia behave in exactly the same manner to both,—forming a gathering in one as in the other.

To a 2 % cane sugar solution therefore the Paramœcia are entirely indifferent, behaving toward it as they would to a drop of the pure fluid in which the solution is made. The sugar solution may be made negative by mixing with it a slight quantity of some substance to which the animals are negative, as NaCl, or positive by adding to it a trace of acetic acid. In all these respects it is exactly like ordinary water.

If we substitute an 8 % solution of cane sugar for the 2 % solution, the phenomena remain almost entirely unchanged. If the 8 % sugar solution is made in distilled water and the Paramœcia are in culture water, they collect in the drop of sugar solution as in distilled water. If the sugar solution is prepared in the same water as that in which the Paramœcia are swimming, they are quite indifferent to it, swimming in and out of the drop without hesitation. If the animals are observed *in* the drop of 8 % sugar solution, however, their conduct is seen to be *slightly* modified. They seem excited, and swim more hurriedly and irregularly than in the pure distilled water. At the same time they may be seen to shrink a little, owing to the withdrawal of water from the protoplasm through the osmotic effects of the sugar solution. These effects are not marked, however.

If the density of the sugar solution is increased to 20 % the Paramœcia behave as follows. On coming to the edge of the drop they make no sign of stimulation, but pass directly in, the motion becoming however a little slower. This is doubtless a mechanical result of the thickness of the sugar solution. Many specimens continue to swim straight ahead very slowly, but before the animal has passed half-way across a drop 6 mm. in diameter it has shrunk to a flat plate, owing to the plasmolysing effects of the dense sugar solution. The animal stops and dies. Other individuals shortly after passing the boundary hesitate, swing the anterior end back and forth, then pass on till they are plasmolysed. Others again after this moment of hesitation *turn* and swim in another direction; some of these thus pass out of the drop and so escape destruction. Still others, after swimming quietly some distance into the drop, stop, then *reverse* the cilia and swim straight backward, often a sufficient distance to carry them out of the drop. But the large majority of all those individuals which, in their random course, swim against the edge of the drop, pass in and are killed by the plasmolysing effects of the sugar solution. In a short time the spot where the drop of sugar solution was introduced is indicated by a large collection of dead Paramœcia.

A drop of 20 % sugar solution is thus incomparably more dangerous to the Paramœcia than a drop of some strong alkali. If a drop of 2 % KOH is introduced under the cover-glass, scarcely a Paramœcium is injured by it, except such as were overwhelmed by the drop as it was introduced beneath the cover-glass. In the case of the alkali the negative chemotaxis of the Paramœcia warns them long before the danger point is reached, so that they swim in another direction, while the sugar solution does not act as a stimulus until plasmolysis has begun and the animal is in too deep to be extricated.

The same results are gained by a study of the conduct of Paramœcia toward dextrose, mannite, glycerine, and urea. Toward solutions of all these substances the infusoria seem entirely indifferent. Solutions of strength insufficient to plasmolyse the animals call forth no reaction whatever. If a drop of very strong solution of any of these is introduced, the Paramœcia enter without hesitation, then show signs of agitation, perhaps reverse the cilia, and finally die of plasmolysis.

The precise conduct of the infusoria toward strong osmotic fluids was studied with especial care in the case of a 10 % solution of glycerine.

After swimming without hesitation some distance into the drop the plasmolytic action begins. The animal reverses its cilia, swimming straight backward some distance, but generally not far enough to carry it out of the drop. The cilia then begin to strike in the original direction again: the animal therefore darts forward. Quickly the cilia are reversed again and the animal swims backward, a somewhat less distance than after the first reversal; then forward a short distance, then backward, the distance covered at each reversal of the cilia becoming less and less. At the same time the animal begins to revolve rapidly on its long axis. Finally the impulses to forward and backward motion seem to become equal; the animal remains in place, revolving rapidly on its long axis, and in a few seconds is dead, the body having shrunken to a flat plate.

Thus while changes in osmotic pressure *do* act as a stimulus for Paramœcium, the stimulus is so weak and slow in action that it does not suffice to save the animals from destruction by plasmolysis. The Paramœcia apparently do not respond until a decided and outwardly evident shrinkage of the cell body has occurred. Tonotaxis seems therefore to play no important part in the life activities of the animal.

The conclusions tentatively expressed earlier in regard to chemotaxis may therefore be now reaffirmed with absolute confidence. Paramœcium aurelia is (1) positively chemotactic toward solutions having a weak acid reaction, (2) negatively chemotactic toward solutions having an alkaline reaction, (3) negatively chemotactic toward *strong* acid solutions, (4) negatively chemotactic toward some neutral salts and some organic compounds, (5) chemically indifferent toward certain organic compounds.

It may be well to give here a list of the substances toward which I have determined definitely the manner of reaction, as they are scattered in the above discussion, and some substances tested have not been heretofore mentioned.

Paramœcium aurelia was shown to be positively chemotactic toward weak solutions of the following substances:—meat extract, rancid olive oil, rancid cod-liver oil, mercuric chloride, copper sulphate, sulphuric acid, hydrochloric acid, nitric acid, acetic acid, tannic acid. All these substances have an acid reaction.

Toward the following, Paramœcium showed itself negatively chemotactic: (*a*) strong solutions of all the substances in the foregoing list; (*b*) *all* effective solutions of potassium hydroxide, sodium hydroxide, sodium carbonate, sodium bicarbonate, sodium chloride, ammonium chloride, potassium bromate, lead acetate, ethyl alcohol and chloral hydrate.

To the substances in the following list Paramœcium is indifferent, so far as the chemical nature of the substances is concerned: cane sugar, dextrose, mannite, glycerine, and urea.

To the list of substances to which Paramœcium is *positive* should be added oxygen, according to the observations of Verworn (1889) and Loeb and Hardesty. According to Israel and Klingmann (p. 327), Paramœcium (aurelia?) is positively chemotactic to water in which metallic copper has lain for a time. The authors do not state definitely whether the "copper water" used in these particular experiments was prepared with distilled water or with the same water as that in which the Paramœcia lived. In the former case the positive taxis observed would probably be due to the distilled water alone.

Regarding the negative chemotaxis of Paramœcium toward CO_2, as reported by Loeb and Hardesty, I shall have something to say in the ensuing part of this paper. It has been shown above that the statement of Loeb and Budget that Paramœcium is negative to acids and alkalies is correct for stronger solutions; these authors themselves state that if weaker solutions are used somewhat different reactions are obtained, though what these different reactions are they do not say.

Determination of the Chemical Substance causing the Collections of Paramœcia under normal conditions.

We are now prepared to investigate the further problem: What is the attractive substance in response to which the Paramœcia gather about the bit of decaying vegetable material in the centre of the slide, as shown in Fig. 2?

As will be recalled, the evidence seemed at first to point to the view that the Paramœcia were positively chemotactic to soluble substances produced by the decaying vegetable material. Later (p. 274) this was shown to be incorrect. The incorrectness of this view may be demonstrated in a most striking manner by a simple alteration in the experiment. Let a bit of *filter paper* or a minute piece of *linen fibre* be substituted for the decaying vegetable material; the Paramœcia gather in a dense collection about it, exactly as about the bit of decaying matter. The experiment may be performed in water from the culture jar in hydrant water or in distilled water; the results are the same.

This experiment raises the question whether the entire foregoing investigation of chemotaxis has not been a movement in a false direction so far as explaining this phenomenon is concerned. If the Paramœcia gather in the same manner about any bit of substance having no active chemical properties whatever does not that prove that chemical action has nothing to do with the phenomenon?

The implication contained in this question is an exceedingly plausible one. However, it may be easily shown to be false. Let the Paramœcia gather about the bit of filter paper, as in Fig. 2. After a time the dense gathering becomes looser; the Paramœcia spread out into a larger clearly defined area, as described in the "Introductory Experiment," and shown in Fig. 3. As will be noticed, the arrangement is much like that shown for the chemotaxis of Paramœcium about a drop of acid substance, in Fig. 8. Now let a fresh preparation of Paramœcia be made, without the bit of filter paper. The Paramœcia are in this scattered uniformly, as shown in Fig. 1.

Next we introduce the point of the capillary pipette beneath the cover-glass of the preparation in which the Paramœcia have formed a group about the bit of filter paper (as in Fig. 3), and remove therewith a drop of water from *within* the area to which the Paramœcia are confined, close to the filter paper. This drop is injected beneath the cover-glass of the second preparation, in which the Paramœcia are uniformly distributed. As soon as a Paramœcium, swimming at random in this second preparation, comes to the drop of fluid injected, it swims directly *in* without hesitation, but on coming to the opposite side of the drop, it stops at once and turns back—exactly as illustrated in Fig. 6, p. 269. It thus remains in the drop, swimming about in the liveliest manner, but never leaving it. Successive Paramœcia are thus quickly collected, and in five to ten minutes all the Paramœcia in the preparation are gathered in the small area where the drop was introduced, exactly as shown in Fig. 5. If a drop of water from some *other* part of the first preparation, outside the area of Paramœcia, is injected into the second preparation, no effect is produced.

Evidently there *is* some substance in the collection of Paramœcia about the bit of filter paper, to which the infusoria are strongly *positively* chemotactic. Furthermore, it is evident that this substance cannot have been produced by the bit of filter paper or linen fibre. It must then have been produced by the Paramœcia themselves.

This becomes apparent also when the mechanism of the collecting together of the Paramœcia about the bit of filter paper is carefully observed. They swim at first hither and thither in every direction throughout the preparation. By chance a few individuals come in contact with the bit of paper or linen fibre. Here they stop (cp. below, p. 298). The small assemblage of Paramœcia thus formed begins to excrete some attractive substance, for the region becomes at once a centre of great attraction for the remainder of the Paramœcia. Moreover, it is not the bit of solid material which is the centre of attraction, but rather the group of Paramœcia, as such. This is proved by the following facts. (1) The Paramœcia will gather in a dense mass about a bit of paper so small that the outer Paramœcia in the mass cannot get within ten times their own length of the paper. (2) In some cases the assemblage begins to take place by chance on one side of the paper. Thereupon all the Paramœcia are attracted to this side: the mass grows in this direction until finally it becomes entirely separated from the bit of paper, which is left quite alone. The latter may then be removed with a needle, while the dense gathering of Paramœcia remains as before. As this is an important matter, an experiment may be given in detail.

9.8 a.m. Preparation *D* made, on a slide, but without a cover-glass. In the centre of the slide an irregular piece of filter paper, with one side smoothly cut, the others torn.

9.24. A noticeable gathering about the bit of paper.

9.44. Almost all the Paramœcia are gathered about the bit of paper. A very large number along the cut edge; many of these cannot get within three Paramœcium-lengths of the paper, yet they crowd in as close as they can.

10.10. The thickest mass of Paramœcia is now at the cut edge of the paper; the outer Paramœcia are at least ten times their own length from it (Fig. 11).

10.20. The Paramœcia have all left the torn edges of the bit of paper, to gather on the cut edge in a single dense mass as large as the entire paper.

10.51. The mass of Paramœcia are now completely separated by a narrow space from the piece of paper; all are pressing toward a centre lying within the group of Paramœcia.

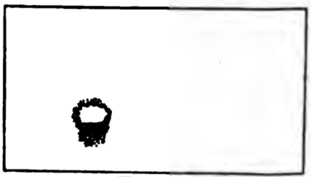


Fig. 11

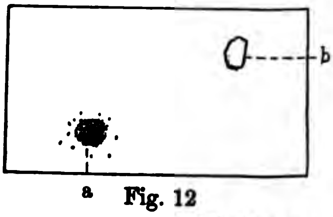


Fig. 12

a, Paramœcia; *b*, bit of paper.

10.53. The bit of paper was removed with the needle from the region of the mass of Paramœcia to the other side of the slide, but the mass of Paramœcia still remains in place (Fig. 12).

The mass of Paramœcia thus entirely separated from the bit of paper was observed for more than half-an-hour longer. It did not loosen and separate into such an area as is shown in fig. 3, because of the absence, in this case, of a cover-glass. (Why this should cause a difference will be explained later.)

It is evident therefore that the Paramœcia are attractive to each other, and the experiment previously described, in which a drop of water from the group of Paramœcia proved attractive to the Paramœcia of another preparation, shows that this must be due to some substance excreted by the Paramœcia and passing into solution in the water. Furthermore, the same substance in somewhat greater concentration produces a negative reaction, since the Paramœcia after a time avoid the centre of the area and gather chiefly about its margin, as shown in Fig. 3.

From what we have learned concerning the phenomena of chemotaxis in general in Paramœcium, we should expect this substance to be an *acid* of some sort. When we inquire what acids may be excreted by Paramœcia, there seem to be two possibilities. The first is that the substance may be the CO_2 excreted in the respiratory process of the animals. The second, that some acid is excreted in connection with the digestive functions. Greenwood and Saunders have shown that an acid is produced in the food vacuoles of infusoria, and have demonstrated that this acid is not CO_2, since it dissolves granules of calcium phosphate. According to the observations of Greenwood and Saunders the acid disappears before the food vacuoles are ejected. However, the possibility suggests itself that a certain amount of this acid persists and is discharged with the waste matter into the surrounding water. Our first approach to a determination of the attractive substance must be by testing whether it can likewise be shown not to be CO_2.

This test was made by introducing granules of finely powdered chalk ($CaCO_3$) into the centre of one of these groups of Paramœcia and observing the effects. The exact form, size and position of a number of irregular granules of the minutest size were noted with the microscope, then the preparation placed in the moist chamber for a number of hours. At the end of this time the granules of calcium carbonate had not suffered the slightest change. On the other hand, a similar trial with the weakest solution of HCl to which the animals showed any sensitiveness produced a marked corrosion of such granules in a much shorter time. The evidence gained in this way is therefore against the presence of any acid stronger than CO_2. What is now desired is a delicate test for CO_2.

At the suggestion of Professor Verworn I used for this purpose a solution of *rosol.* This produces with water a beautiful red solution, which is decolorized by carbon dioxide—as well as by other acids, of course. A weak solution of rosol is not injurious to the Paramœcia, so that a large number of the infusoria were brought directly into the solution, and a preparation was made with this. A bit of filter paper was placed in the centre, and the Paramœcia formed a dense gathering about it, as usual.

Shortly after the collection has become densest the solution begins to become colourless about the bit of paper. The group of Paramœcia begins to loosen and separate, and the colourless space to become larger. The Paramœcia remain confined to a definitely limited area (as shown in Fig. 3) which continually but slowly grows larger; the colourless area is exactly identical in size and limits with this area to which the Paramœcia are confined and holds exact pace with it in its increase in size. If the preparation be now viewed with a dark background the Paramœcia are seen distinctly as white dots gathered in a definitely limited area, the greater number of Paramœcia about the outer boundary of this space. If the same preparation be viewed with a white background the Paramœcia disappear, and one sees only the clear red colour of the greater part of the slide contrasted with the entirely colourless area in the centre, of exactly the form and size of the area previously seen to be marked by the Paramœcia. The reaction is a most striking, precise and satisfactory one, and, taken in connection with the lack of reaction towards $CaCO_3$, clearly indicates the presence of CO_2 in the area.

There remains but to test CO_2 directly. Pure carbon dioxide was made by the action of HCl upon $CaCO_3$. The gas was collected over water and washed carefully by shaking it up with a solution of sodium carbonate. The tests were made in exactly the same manner as for other chemical substances. A preparation was made in which the animals were scattered uniformly beneath the cover-glass; then a bubble of carbon dioxide was introduced with the capillary pipette. Immediately before or after, a bubble of air was brought into another part of the same preparation as a control.

The result of the experiment is not doubtful for a moment. The

Paramœcia are strongly positively chemotactic to the carbon dioxide. They gather at once in a dense collection about the bubble of CO_2, pressing against the very walls of the bubble and crowding each other to get as near as possible. Meanwhile the bubble of air is left entirely alone.

I give herewith three figures from my record of experiments, illustrating the chemotaxis towards CO_2. The three figures are from a preparation of Paramœcia in distilled water. A bubble of air (*a*) was introduced near the left end of the cover-glass, a bubble of CO_2 near the right end. Figure 13 shows the preparation two minutes after the bubbles were introduced. Many Paramœcia are crowded close about the bubble of CO_2.

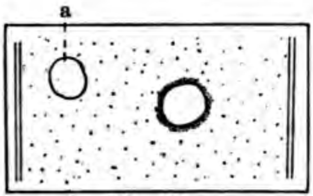


Fig. 13

Chemotaxis of Paramœcia toward CO_2. Two minutes after the introduction of the bubble of CO_2.

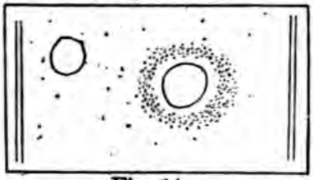


Fig. 14

Two minutes after Fig. 13.

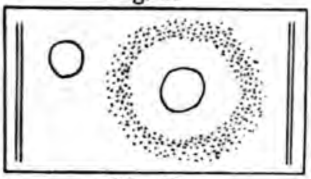


Fig. 15

Same preparation 18 mins. later than Fig. 14.

The condition two minutes later is shown in Fig. 14. The gathering of Paramœcia about the CO_2 has become larger, but the animals are not crowded so close to the bubble; there is a distinct space between it and the dense ring of Paramœcia. They are evidently *negative* toward a too concentrated solution of CO_2.

Fig. 15 shows the condition 18 minutes later. The ring is broad and not so dense as before; the inner boundary is much farther from the surface of the bubble than in Fig. 14. The Paramœcia in the ring are exceedingly active, generally swimming around the bubble in a circular path.

A large number of such experiments were performed and always with the same result. The experiments may be performed with the Paramœcia in distilled water, in culture water, or in hydrant water: the results are the same. We must conclude therefore that Paramœcium aurelia is positively chemotactic toward a solution of CO_2 of a certain strength; negatively chemotactic toward a stronger solution. The positive chemotaxis toward CO_2 seems much more powerful than towards other substances, since Paramœcia which had been brought only the day before into distilled water, and showed almost no recognizable positive chemotaxis toward other substances, were at once strongly attracted by a bubble of CO_2.

The positive chemotaxis toward CO_2 may be easily shown in another manner. If a bubble of ordinary breath is blown beneath the cover-glass, with a capillary tube, the Paramœcia are distinctly positively chemotactic to it, though not so powerfully so as towards pure CO_2. The experiment will of course not succeed unless the breath is blown through the tube sufficiently beforehand to be sure that all ordinary air is removed from it before the experiment is made.

As will be noticed, the phenomena observed with CO_2 are exactly the same as those observed with the substance excreted by the Paramœcia, and which we had concluded on other grounds was probably CO_2. A drop of water can be taken from within the ring, in the one case as in the other, and this drop proves strongly attractive to the Paramœcia of a fresh preparation. If the electric current be passed through the preparation shown in Fig. 14, the Paramœcia all gather on the cathode side of the ring, but go no farther toward the cathode, exactly as was described in the case of the preparation shown in Fig. 3. There can be no question that we are dealing with carbon dioxide in both cases.

Armed with this knowledge, we can now explain a number of exceedingly puzzling phenomena which have already been mentioned in this paper or elsewhere.

Explanation of previously observed phenomena by means of the reaction toward CO_2.

We may first summarize briefly the essential features of the solution of our original problem—the course of events in the "Introductory Experiment" (p. 260). A certain number of Paramœcia strike by chance against the bit of decaying vegetable material, and remain there. The water in this region then becomes more strongly charged with CO_2 than elsewhere, owing to its excretion here by this collection of Paramœcia. This region then becomes a centre of attraction for other Paramœcia, owing to their positive chemotaxis toward CO_2; so all collect here. The production of CO_2 is thereby so increased about the bit of vegetable material that the solution in the water here becomes too strong: the Paramœcia become negative to the CO_2 and leave the centre of the group. They go only to that point where the concentration of CO_2 is that to which they are positive, however, and here they remain, on the margin of the area. Individual differences exist in the sensitiveness to CO_2, so some Paramœcia will be found in the centre of the area after most are gathered at its margins. The continued growth of the area is due to the continued excretion of CO_2 by the Paramœcia. If the constant electric current is passed through the preparation, the Paramœcia react to the electric stimulus normally till this reaction comes in conflict with the chemotactic reaction, which occurs at the cathode side of the area; the chemotactic proving generally the stronger, the Paramœcia do not leave the area.

A phenomena related to the above was described and figured by Jensen. When Paramœcia are brought upon a slide, beneath a cover-glass, they soon begin to avoid the outer edges of the water, not swimming within a certain distance of the margin. The free margin thus left becomes broader and broader till the Paramœcia are confined to a comparatively small central space. Jensen explained this as probably due to the greater concentration of the salts in the water about the edge, due to the evaporation here. It seems much more probably due to the discharge of the CO_2 which is being continually produced by the Paramœcia, into the air about the edge of the cover-glass, leaving this region much poorer in CO_2 than the inner part. The Paramœcia begin to gather on this account into the central region—whereupon the difference in the amount of CO_2 between the outer and inner regions becomes greater than ever, tending still more to accentuate the gathering of the Paramœcia in the central region. That this is the true explanation is rendered especially probable by the fact, at least when a large cover-glass is used, that the collection does not always take place under the middle region of the cover-glass, but may occur in almost any part, the precise place where the assemblage occurs being determined by chance factors. Such a "chance factor" is a bit of filter paper or linen fibre. A slight roughening of the surface of the glass, or the presence of the merest trace of some attractive substance may determine the place of gathering in the same manner. If there is no such determining factor, the Paramœcia simply avoid the outer edges and gradually gather in the centre, as described by Jensen. The water from one of these central collections of Paramœcia is attractive, on being introduced into a fresh preparation. This is of course inexplicable on the theory that the gathering together of the Paramœcia is due to the concentration of salts about the margin of the preparation. If after such a central group has been formed, the electric current is passed through the preparation, the Paramœcia respond only in so far as the electrotaxis does not come in conflict with chemotaxis. This explains why, as is often noticed, the Paramœcia of a preparation may at first respond typically to the electric current, while later the Paramœcia of the same preparation respond but incompletely or scarcely at all.

In close relation to this stands the cause of the fact mentioned on page 277, that in testing various solutions with Paramœcia on the slide, it is necessary to make the tests at once after the Paramœcia are brought into the preparation, or the results are unreliable. After remaining some time the Paramœcia will have gathered into a group in some part of the slide, and will there have produced a certain amount of CO_2. Being more strongly positive to this than to any substance which can be introduced, they may now show negative taxis toward the solution tested, when previously they would have reacted positively to the same solution.

A related phenomenon is the following. On page 271 it was stated that if Paramœcia are removed from a dense collection in the culture jar by means of a pipette and brought at once beneath the cover-glass of a preparation, they show themselves negatively chemotactic to all substances introduced, of whatever nature. In such a case the Paramœcia do not remain even a short time uniformly distributed beneath the cover-glass, but will be found gathered in some particular region of the slide a few seconds after they are introduced. Fig. 16 shows a preparation made in this manner; the Paramœcia are gathered in a long irregular strip near one side. The explanation is as follows. In the dense collections of Paramœcia in the culture jar

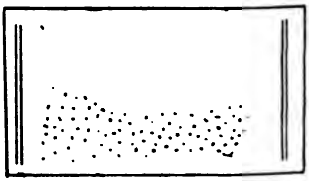


Fig. 16

the water contains in solution a large amount of CO_2 excreted by the Paramœcia. These collections are so small that in removing Paramœcia from them with a pipette water from outside the collection, containing little CO_2, is drawn into the pipette also. The whole is now brought at once under the cover-glass without a thorough mixture having taken place; the Paramœcia betake themselves at once to that part of the water containing more CO_2, leaving the rest of the slide empty. Now, if some substance is introduced beneath the slide, to which the Paramœcia are generally positively chemotactic, they do not respond positively, being more attracted by the CO_2. If however the water from the culture jar is thoroughly stirred up with the pipette and brought in contact with the air before being placed on the slide, the CO_2 is driven off, and the conduct of the Paramœcia is not therefore modified by its presence; they give the normal positive reaction to the solutions introduced.

On page 286 it was stated in describing an experiment, that the Paramœcia, having gathered in a dense mass, did not later loosen and separate, on account of the absence, in this experiment, of a cover-glass. The reason for this is easily seen when one considers the effect of a cover-glass in preventing the escape of carbon dioxide which is produced beneath it. If no cover-glass is present, the CO_2 continually escapes, so that the water solution does not increase in strength beyond that concentration which is attractive to the Paramœcia; therefore they continue to crowd together in order to reach the point where the CO_2 is densest. If the CO_2 in the thin layer of water is prevented from escaping by the cover-glass above it, the solution becomes more and more concentrated till it reaches a strength to which the Paramœcia are negatively chemotactic; hence they separate, remaining chiefly about the margins of the CO_2 area. It is probable that under natural conditions the CO_2 hardly ever reaches such a concentration as to be repellant, owing to its continual diffusion and escape. Thus while the positive chemotaxis of Paramœcium toward CO_2 plays a very large part in the everyday life of the infusorian, it is probable that the negative chemotaxis toward a denser solution comes hardly ever into operation.

Certain phenomena described by Pfeffer (p. 618) in other infusoria than Paramœcium probably receive their explanation also by the attraction of the infusoria toward the carbon dioxide excreted in a dense group. Pfeffer observed that when numbers of *Glaucoma scintillans* are brought upon a slide, together with some small neutral solid bodies, such as bits of filter paper, the infusoria quickly gather into dense groups about the solid bodies. Such large and dense gatherings were formed and there was such variation as to which particular bit of the solid should form the centre of a group, that Pfeffer felt that the phenomenon could not be explained by the contact stimulus exercised by the solid alone, and was inclined to believe that it was partly due to a contact stimulus resulting from the striking together of the infusoria in such a collection. Similar collections in a less pronounced degree were observed by Pfeffer in *Colpidium colpoda,* and still less marked in *Stylonychia mytilus* and in *Paramœcium aurelia* itself. As we know that the collections are due in the case of Paramœcium to the attraction toward the CO_2 excreted in a dense group, the conjecture is exceedingly plausible that the same cause accounts for the phenomena in the other cases.

If a quantity of water containing many Paramœcia is brought into an open watch-glass or other small vessel, the infusoria are at first distributed uniformly. Very soon, however, the Paramœcia begin to avoid the upper surface and gather toward the bottom. Soon a distinct boundary is evident between the water containing Paramœcia and that containing none. The Paramœcia continue to draw together toward the bottom until at last a small definitely bounded space in the bottom of the watch-glass alone contains Paramœcia. The individuals may be seen to swim straight forward within this space, until, coming to the invisible boundary, they are turned back. This is evidently a phenomenon of the same nature as those above described. The CO_2 produced by the Paramœcia is given off at the surface of the water: therefore the Paramœcia draw away from the surface. They continue to draw together until a balance is struck between the amount of CO_2 produced and that given off; they remain confined to the space in which the CO_2 reaches that concentration to which they are positive. A drop of water taken from this gathering in the bottom of the watch-glass proves attractive when introduced into a slide of fresh Paramœcia. If two unpolarisable electrodes are dipped into the water on opposite sides of a watch-glass containing such a group of Paramœcia in the bottom, and the circuit is closed, the Paramœcia may be induced by the electric current to swim from one side of the small bottom group to the other, but only with the greatest difficulty can they be forced across the boundary of the group into the surrounding water. This furnishes the explanation of the fact often noticed that in order to demonstrate clearly the phenomena of electrotaxis in a watch-glass of

Paramœcia, either the circuit must be closed soon after the infusoria are brought into the glass, or the water must be thoroughly stirred up occasionally.

If in place of a concave watch-glass a vessel is used having a broad flat bottom, the Paramœcia gather in one or more well-defined groups in some particular part of the dish. They do not scatter all over the bottom, as must be the case if the collecting together were due to simple concentration of the salts in the upper layers of the water, owing to evaporation. Exactly *where* the Paramœcia shall gather depends on chance circumstances; a slight variation in the warmth of the two sides; the presence of a small solid object in one spot, or a roughening on the surface of the glass. Anything which leads in the beginning to a collection of a few individuals in a certain region, results later in the gathering of all (or most) of the Paramœcia in the vessel in this place, owing to the greater concentration of CO_2 in this region. The original occasion for the collection may then be removed, but the collection itself remains.

On p. 276 it was stated that if Paramœcia which are to be used in experimentation are placed in a beaker, and are found by testing to be *positive* toward distilled water, they may later become *negative* to it unless well stirred up at short intervals—thus causing the series of experiments to give inconstant and worthless results. This is explained by the observations just described. If left untouched for some time, the Paramœcia gather together in some part of the vessel and excrete CO_2, to which they are strongly positive. If now, some of these Paramœcia, in this water strongly charged with CO_2, are brought upon the slide and tested with distilled water, they avoid the latter, on account of their *greater* positive chemotaxis toward CO_2. If the carbon dioxide is driven out of the water by stirring it and bringing it into contact with the air, the Paramœcia gather in the distilled water. In the same way the chemotaxis may vary toward other substances.

The explanation of the fact noted on p. 273, that Paramœcia may become positively or negatively chemotactic to water from a different part of the same dish as that in which they are swimming, lies along the same line. If Paramœcia taken from a part of the dish where the water contains much excreted CO_2 are tested with water from a part poor in CO_2, the Paramœcia are negative; in the converse experiment they are positive.

The place where the Paramœcia will form a permanent gathering in a shallow vessel may sometimes be determined artificially by the use of the electric current. As is well known, when a constant electric current is passed by means of non-polarisable electrodes through a vessel of water containing Paramœcia, the latter gather *behind* the cathode. The explanation why they gather behind the electrode rather than in front of it or upon it was given in principle by Ludloff, though he did not apply the principle to this particular problem. He showed that the movement toward the cathode was due, not to any attraction of the cathode, but to the effect of the current in orienting the animal with its anterior end toward the cathode. The animal then swims in that direction, but there is no reason why it should stop on arriving at the cathode. On coming to the latter, the animal is as it were left to itself, and can swim where it pleases, the slight current components behind the electrode being ineffective so far as the Paramœcia are concerned. If the animals return into the space between the two electrodes, they are at once brought back as far as the cathode, then left to themselves again. Thus there is naturally produced finally an assemblage of Paramœcia behind the cathode. This may be very well demonstrated by performing the experiment in a long trough, with the cathode in the middle of the trough, the anode at one end, and all the Paramœcia at first between the two electrodes. The Paramœcia are soon all brought by the current into that half of the trough lying behind the cathode, where they swim about at random, paying no further attention to the electric current. If the experiment be performed with the cathode close to the wall of the vessel, so that only a small space is left behind the electrode, the Paramœcia gather in a dense mass in this small space. If a rather weak current is used and this is long continued, the Paramœcia may settle themselves against the wall of the vessel, and remain there after the electric circuit is opened or the electrodes removed, in consequence of their positive chemotaxis toward the CO_2 produced in the dense assemblage. This experiment does not always succeed, as the electric current often produces such excitement in the Paramœcia that on being released from its action they swim in every direction from the spot where they were collected. This is especially likely to happen if the gathering has lasted but a short time, so that little CO_2 has been produced. Moreover, if the gathering of Paramœcia so formed lies near the upper surface of the water, the CO_2 may be dissipated as fast as it is produced, so that the Paramœcia do not remain after the circuit is opened.

Jensen (p. 438) has described the conduct of Paramœcia when left in an upright open tube of water. They rise to the upper end of the tube, on account of their negative geotaxis. But after a time they draw back from the surface, leaving first the small elevated columns of water about the margin of the concave upper surface, later sinking even lower, and presenting a clearly defined upper boundary, which they do not pass. (See the Figs. II. III. and IV. of Jensen.) This phenomenon may likewise be explained by the escape of carbon dioxide from the upper layers of the water. The Paramœcia sink and gather together until their own production of carbon dioxide suffices to supply the loss due to the escape of the gas from the upper surface of the water, remaining at the point where the charge of CO_2 is that to which they are most attracted.

It was stated on page 265 that the method of testing the chemotaxis of Paramœcium toward various substances by introducing capillary tubes containing the solution to be tested into a vessel containing many Paramœcia and leaving them there for some time is unreliable. One reason for this is as follows. After some time the Paramœcia may occasionally be found to have gathered into tubes containing substances to which they are entirely indifferent, as for example, water of the same sort as that contained in the rest of the vessel. This is evidently due to the following. Certain Paramœcia stray by chance into the tube and there of course produce CO_2, which is prevented from diffusing. Others accidentally entering the mouth of the tube are attracted by this CO_2 and likewise remain. Thus the production of carbon dioxide increases till the gas perhaps begins finally to diffuse about the mouth of the tube. Thereupon this becomes the centre of attraction for other Paramœcia, till at last all the animals in the dish are gathered in an assemblage about the mouth of the tube or within it. We may say then that the fact, taken by itself, that Paramœcia gather in a certain region is no evidence that the region previously contained substances attractive to the animals.

In general, it will be found that very many of the phenomena presented by the behaviour of Paramœcia in fluids are explicable on taking into consideration the following factors; (1) the production of CO_2 by the Paramœcia: (2) the positive chemotaxis of Paramœcium toward CO_2 in a certain concentration: (3) the negative chemotaxis of Paramœcium toward a higher concentration of CO_2 (much less important than the foregoing); (4) the diffusion and escape of CO_2 in the water. In all experimental studies on reaction to stimuli in Paramœcium, close attention to these factors is an absolute necessity, if reliable results are to be obtained.

The explanation of these phenomena as due to the positive chemotaxis of the organisms toward carbon dioxide is of course a reversal of the reasoning commonly used, in which explanation is sought by assuming that organisms are always repelled by CO_2. Such a reversal will doubtless be met with criticism and opposition on first thought.

3. Thigmotaxis.

It has been shown above (p. 287 *et seq.*) that the chief cause of the gathering of Paramœcia around a piece of paper or linen is the production of CO_2 by the Paramœcia themselves and their positive chemotaxis toward this substance. We must now investigate the exact part played by the solid body.

Dewitz showed that a tendency to cling to and move along solids plays an important part in the life activities of the spermatozoa of the cockroach. A similar fact was made known by Massart (1888 and 1889 *a*) for the spermatozoa of the frog. Pfeffer (p. 618) observed collections of *Glaucoma* and other infusoria about bits of filter paper or other neutral solids, and referred these collections to the stimulus of contact. Verworn (1889, p. 90) introduced for this directive stimulus due to the action of contact the name Thigmotropism; in accordance with the terminology used throughout this paper I shall employ the word thigmotaxis. Verworn (*l.c.*) pointed out in a general way that positive thigmotaxis plays a part in the movements of diatoms, oscillaria, and hypotrichous infusoria, and that negative thigmotaxis was to be recognized in the sudden backward movements of Flagellates and Ciliates on striking solid objects, as well as in the retraction of the pseudopodia of rhizopods on being subjected to a mechanical stimulus. Loeb (1890) studied the stimulus produced by mechanical contact in a number of higher animals, and gave to the phenomena which he observed the name *stereotropism.*

Any small object of character similar to filter paper acts equally well as the centre of a gathering of Paramœcia. A piece of any other paper, a small bit of linen or other cloth fibre, a bit of sponge, etc., all have the same effect. Any of these substances may previously be boiled in the same fluid in which Paramœcia are found, in order to remove all traces of oxygen, carbon-dioxide or any other soluble substance which might be contained within it; yet the effects are the same. The experiment may be performed with the Paramœcia in hydrant water, in water from the culture jar, or in distilled water, without altering the result.

There can be no question therefore that the bit of paper or other body acts merely in virtue of its being a solid of a certain physical structure, and not in consequence of any chemical action, in determining where a collection of Paramœcia shall take place.

The beginning of a collection about such a body, possessing as it does no influence at a distance, is made possible by the continuous motion of the Paramœcia. If a preparation of Paramœcia on a slide, containing in one spot a small bit of filter paper is closely observed, the Paramœcia are seen at first to swim hither and thither in every direction, apparently without directive tendency of any sort. They swim now in a curved path, now in a straight one, sometimes about the margin, or again cutting across the middle. Soon a single individual strikes in its headlong course the bit of paper. It stops at once, often starts backward a slight distance, and whirls on its short axis two or three times, then settles against the bit of paper, and remains. Quickly another and another strike in the same way and remain. Now the excretion of CO_2 by the animals gathered together begins to take effect; the region becomes a strong centre of attraction, and in ten to fifteen minutes, and often less, the paper is surrounded by a dense swarm of Paramœcia, containing a large majority of all those in the preparation. Of course only the beginning of the collection is to be considered due primarily to the reaction to solids; the remainder of the phenomenon comes under the head of chemotaxis.

The habit which the Paramœcia have of swimming swiftly hither and thither, often changing the direction of the course, is evidently essential to the occurrence of the thigmotactic effects. In the preceding part of the paper it was shown to play likewise an important part in chemotaxis. The continual and rapid change of position of the body of the animals subjects them to many more stimuli than would otherwise fall to their lot, and brings every individual in a preparation in a comparatively short time into the region of influence of any stimulus the direct action of which is confined to only a limited area.

Thigmotaxis alone is not capable of producing the dense assemblages of Paramœcia above described. If a quantity of water containing Paramœcia is placed in a flat-bottomed open dish, and a bit of filter paper or other similar solid introduced, the Paramœcia commonly form no collection about it. If such a solid is watched with the microscope it will be found that the behaviour of a Paramœcium on striking it by chance is at first exactly as toward the bit of paper under a cover-glass. The Paramœcium applies itself to the paper and remains. But the CO_2 continually escapes, so the place does not form a chemotactic centre, and in a short time the Paramœcium swims away. There is much chance in this matter, however: if other causes combine to make this particular region a centre for the Paramœcia, then sufficient CO_2 is produced to supply the escape, and the Paramœcia *do* gather on the bit of paper. For example, this may be the case if the solid body lies in the bottom of a convex watch-glass (cp. p. 294).

The Paramœcia do not gather with equal avidity about solids of all sorts; the body must be of a certain type of physical structure to excite easily the thigmotactic reaction. *Fibrous* bodies, such as frayed filter paper, are especially effective. Materials composed of light particles have a similar effect; thus Paramœcia quickly swarm upon a mass of powdered carmine when it is introduced beneath the cover-glass. Starch grains act in the same way. Also if saliva be mixed with the water, the Paramœcia swarm about the remains of the dead cells contained in it, though to filtered saliva they are negatively chemotactic. Surfaces covered with a layer of sticky mucus-like material are likewise very effective; a bit of glass which has been dipped in saliva or white of egg is soon surrounded by the infusoria. This reaction is not due to positive chemotaxis, since if the Paramœcia are tested with albumen or saliva alone, they are found to be indifferent or negatively chemotactic. It is not however a matter of mechanical fastening to the surface by being caught in the sticky substance. The Paramœcia are entirely free in their motions, shifting their positions, leaving and returning and acting in all respects like the individuals gathered about fibrous or powdery bodies.

To smooth hard bodies the Paramœcia do not respond unless chemotaxis is combined with thigmotaxis. Perfectly clean glass, stone, wood, and the like rarely become the seat of such swarms as above described.

The tendency to gather about and settle upon solids is strikingly increased by the presence in the surrounding water of some substance to which the Paramœcia are positively chemotactic. This is especially true when the water contains the proper amount of CO_2. For example, as just stated the animals do not commonly tend to gather on smooth hard substances, such as the glass tubes generally used in my experiments for supporting the cover-glass. Suppose, however, that in the experiment with CO_2 shown in Figs. 13 to 15 (p. 289) the bubble of gas is situated close to one of the glass rollers used in supporting the cover. As the ring of Paramœcia spreads out, it will at perhaps the stage shown in Fig. 14 come in contact with the glass roller. Thereupon the Paramœcia in that part of the ring settle upon it. As all the Paramœcia in the ring swim around the bubble in a circular path, soon almost all will have come in contact with the glass roller and may settle upon it, till there is nothing left of the ring of Paramœcia except the spot where it was tangent to the glass roller. As the CO_2 continues to spread the circular area becomes larger, so that the glass roller cuts the circle in two points instead of being tangent to it. The Paramœcia follow the circumference of the circle as it moves along the glass rod, the original simple group of attached Paramœcia separating into two, which move farther and farther apart along the rod as the circle becomes larger. A stage in such a preparation is shown in Fig. 17.

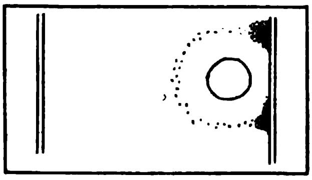


Fig. 17

Within the area formed by the excretion of CO_2 by the Paramœcia (such as shown in Fig. 3), it can be observed that, even if no large solid body is present about which the Paramœcia may gather, many of them will have their anterior ends applied to small particles in the water, or numbers of them are collected about roughened spots on the surface of the glass slide. The increased tendency to react to the contact stimulus in the presence of CO_2 is observable in many ways.

The phenomena of thigmotaxis may be exhibited toward bodies of extraordinarily minute size. This is shown by such observations as the following. A single carmine grain, of not more than $\frac{1}{8}$ the diameter of the short axis of a Paramœcium had become fastened by a minute fibre of some sort in such a way that it was movable, but could not be carried away from the region where it was anchored. Paramœcia were numerous in the preparation, and occasionally one would strike with its anterior end against the grain of carmine. Thereupon it would stop with its anterior end applied to the grain, and begin to swing in a circle about the latter, pushing it hither and thither, but not being able to loose it from its moorings. This continued for a minute or more, the energetic ciliary motion nicely adjusted in such a way as not to destroy the contact with the grain. After a minute or two the Paramœcium left the grain. Soon another individual struck it and the performance was repeated. Occasionally two or even three would gather about the grain at the same time, all swinging together in such nice adjustment that none were displaced.

Very often one notices on a slide containing many of the infusoria numerous small groups of Paramœcia, consisting of half-a-dozen individuals, more or less, having their anterior ends applied together, and the whole group revolving and progressing like a colonial infusorian. I have examined carefully many such cases and have never failed to find at the centre of such a group a minute particle of some sort, against which the anterior ends of the Paramœcia were pressed. Such observations show an extreme sensitiveness to contact in the anterior ends of the Paramœcia.

In a similar way it will often be observed that a Paramœcium is carrying about, at its anterior end, a single minute grain of some substance. Under such circumstances the infusorian exhibits extremely characteristic motions (cp. p. 304).

Position of the Animal and Motion of the Cilia in Thigmotaxis.

In the case of individuals gathered about a larger solid body, such as a bit of paper or a piece of decaying plant tissue, the anterior end is commonly applied to the surface of the solid, as just described in the case of minute granules. This is not by any means always the case, however; almost any part of the resting individual may be applied to the solid. For example, in the case of fibrous bodies, the animal may often be seen to lie lengthwise along a fibre, touching it only on one side, while both ends are free (Fig. 18). In other cases a Paramœcium may lie crosswise to a fibre, or be touched in two different regions by different fibres.

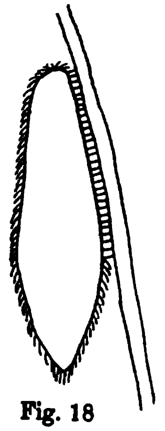


Fig. 18

There seems to exist a typical arrangement and motion of the cilia in individuals exhibiting thigmotaxis, but this arrangement is not invariable, and a great variety of motions may occur in thigmotactic individuals. It will be important however to gain a conception of what this typical condition is, both for its own sake, and because of its significance for an understanding of the peculiar interference of thigmotaxis and electrotaxis, to be described below.

For this purpose it is impossible to use the method employed by Ludloff for studying the motions of the cilia in electrotaxis,—the introduction of the animals into a thick gelatine solution. In such a solution they are not under normal conditions and do not respond to the contact stimulus, at least not in a pronounced and normal way. The motion of the cilia must therefore be observed under normal conditions in ordinary water. Direct observation of the cilia in this way presents great difficulties: it can be used to a certain extent as a control for other methods, but the most satisfactory results may be obtained by observing the currents produced by the cilia, shown by the motion of particles in the surrounding water. As such particles the bacteria in the water in which the Paramœcia live may be employed. The clearest results however are gained by the well-known method of introducing powdered carmine into the water.

Using these methods we find the typical arrangement and motion of the Paramœcia in thigmotactic individuals to be as follows:

The cilia in that region which is applied to the solid body are entirely motionless. They are not pressed down against the body of the animal, but appear like minute straight stiff rods, attaching the Paramœcium to the object (Fig. 18). Where the latter is very small, as a grain of carmine, the few cilia touching it gain the appearance of stiff prehensile organs, used for holding the object. If the body is large and soft, as in the case of the decaying plant tissue commonly used as feeding grounds by the Paramœcia, the anterior end is imbedded for a certain distance in the soft material, and the cilia in this whole imbedded region are stiff and quiet.

In the oral groove the strong cilia are exceedingly active, all striking toward the mouth. Within the groove the cilia are therefore directed toward the rear, producing a strong backward current of the carmine particles. On the elevated edges of the groove the cilia strike inward, toward the middle of the groove, so as to drive particles into the current passing along the groove toward the mouth. Behind the mouth the strong current passing backward is stopped and turned outward, away from the animal. The result is the formation of a strong whirlpool on the oral side of the anterior half of the infusorian, as shown in Fig. 19. (Whether this strong motion of the oral cilia is to be considered in itself a reaction to the contact stimulus, or whether these cilia move in the same manner in the free swimming animal I have not determined.)

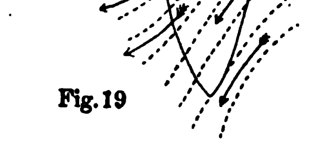


Fig. 19

Side view of Thigmotactic Paramœcium, showing currents of water.

Over the remainder of the body of the animal the cilia are comparatively quiet. They are not held stiffly immobile, as at the part in contact with the solid body, but may generally be seen to be quivering everywhere. Occasionally in one or the other part of the body the cilia are exercised energetically, changing the position of the animal, forcing it closer to the object or the like. In cases where several individuals are gathered about a single movable particle, as described earlier, the cilia covering the body (which may be distinguished as *locomotor* cilia as compared to the *oral* cilia in the groove), must of course be in active co-ordinated motion. The characteristic effect of the contact stimulus on the locomotor cilia is to inhibit their motion: they may however respond to a certain extent to other stimuli, while in this condition, as will be shown in detail for the electric stimulus.

As the locomotor cilia are thus comparatively still, they do not produce currents when the animal is quietly feeding; the entire set of characteristic currents under this condition is due to the whirlpool caused by the cilia of the oral groove. The water rushes in from all sides to join this whirlpool, so that the currents present an arrangement which is shown in side view in Fig. 19. From the anterior end and the aboral side the currents pass obliquely toward the mouth, and then to the rear, so that everywhere at the posterior end of the animal the currents are to the rear. This is modified in some cases, probably by the position of surrounding objects, in such a way that the currents at the posterior end form an arch, obliquely forward on the aboral side, obliquely backward on the oral side. Either of these arrangements is comprehensible as due to the presence of the whirlpool on the oral side of the anterior end. In the former case the water forming the currents which pass into the whirlpool from the aboral side comes more from the anterior end; in the latter case more transversely or partly from the posterior end,—the anterior region being blocked by the presence of a solid body.

When the solid body inducing the thigmotactic reaction is very small and movable, as in the case of a single carmine grain at the anterior end of the animal, the Paramœcium does not itself remain quiet, but moves in a peculiar manner. The creature moves partly forward, partly sideways, in such a way that it describes a circle, or rather, its two ends describe two concentric circles, the inner one being traced by the anterior end; the long axis of the infusorian always lying in a radius of the two circles. Reflection shows that this motion is merely the resultant of the various directive impulses due to the motion of the cilia in the oral groove, as indicated in Fig. 19. The cilia beating strongly backward in the oral groove of course impel the animal forward. But as the active cilia are all on one side, there is also a tendency to move toward the opposite side. The resultant of these two motions at right angles to each other is a motion in the circumference of a circle. In fact, the animal moves forward in exactly the lines indicated by the arrows in Fig. 19, only of course in the opposite direction from the water currents. The ciliary motion is thus the same whether the thigmotactic animal is at rest or in motion: in the former case it is the water currents that move obliquely backward; in the latter case the animal moves on the same lines obliquely forward. The Paramœcium is, as it were, whirled about in its own whirlpool.

Summing up, we may say that under the influence of the contact stimulus the cilia of three regions of the body are affected in three different ways. The cilia which are in contact with the solid body are held stiff and immobile, at right angles to the surface of the animal. The cilia of the oral groove have a strong motion directed toward the mouth. Over the remainder of the body the cilia are quiet or quiver ineffectively.

Interference of Thigmotaxis and Electrotaxis.

As described in the "Introductory Experiment" (page 260), after the Paramœcia have gathered closely about the bit of decaying plant tissue or other solid, they no longer respond to the electric current. Before collecting about the bit of paper, the Paramœcia respond to a current from five or six or even a less number of chromic acid cells. After they have collected on the solid, the same current seems not to affect them. Increase the current to 10 cells; 20 cells; the Paramœcia still remain quietly against the bit of paper. When 30 cells are employed the current is so strong that sparks are obtained as the quicksilver key is closed, and it passes through a very thin layer of water containing the Paramœcia, so that the intensity must be great. Still the Paramœcia remain in place. Very slowly, however, the outermost Paramœcia in the group may now be seen to loosen themselves and swim to the cathode,—especially if the direction of the current is reversed several times in succession. But many maintain their position for a long time. With a weaker current—say of 10 cells—the Paramœcia retain their position indefinitely, though under other circumstances they respond at once and strongly to such a current.

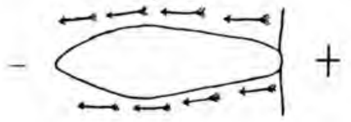
We know from the work of Ludloff that the effect of the constant electric current upon Paramœcium is due to its influence on the stroke of the cilia. At the side or end of the Paramœcium which is directed toward the anode the cilia are so influenced that the effective stroke is to the rear, and urges the animal forward. On the cathode side or end the influence is such as to make the effective stroke of the cilia forward, thus urging the animal itself backward. The result is that if the animal lies in a position which is oblique to the direction of the current, it is at once turned by the action of the cilia above described, until it lies with its long axis in the direction of the current and its anterior end to the cathode. The animal then swims to the cathode as a result of the greater effectiveness of the backward stroke of the cilia on the posterior (anode) half of the body, as compared with that of the forward stroke on the anterior (cathode) half. (For details see the paper of Ludloff.)

The key to the fact that thigmotactic Paramœcia do not respond to the electric current must be looked for therefore in some change in the way that the cilia react to the electric stimulus. I have studied this reaction in the same manner as above described for the study of the ciliary motion in thigmotaxis alone. Carmine grains were introduced into a preparation of Paramœcia in which the animals were gathered about some solid object, then the currents of water produced by the motion of the cilia when the constant electric current was passed through the preparation were observed. For applying the unpolarisable electrodes to the preparation, the cover-glass was supported at each end either by pieces of filter-paper, or by disks of clay, as described by Ludloff.

The necessary observations require a great deal of time and patience, as individuals under exactly the required conditions are not easy to find. The Paramœcium to be observed must of course lie in the characteristic thigmotactic position, resting against some solid body. Furthermore, it must be a solitary individual, at some distance from others, in order that the currents produced by its cilia may not be interfered with by currents from other Paramœcia. As the Paramœcia tend to congregate, this condition is hard to find fulfilled. Then the individual must lie either parallel with or transverse to the direction of the electric current, as obliquely lying individuals do not give clear results by the method of studying the currents. Having found such an individual, it is next necessary to observe carefully the direction of the currents caused by the cilia before the electric circuit is closed, as well as to note which end or side is toward the future anode or cathode.

We will take up first the case where the Paramœcium lies with its long axis in the direction of the electric current, the anterior end to the *anode*, the posterior to the cathode. The anterior end of the animal is pressed against the piece of filter paper, and the currents of water are as shown in Fig. 19. As will be seen from this figure, in the commoner case the current is obliquely to the rear over the whole animal, and if the infusorian is seen in any but a side view this obliqueness disappears, the currents seeming to pass straight back. The position of the animal and direction of the currents may therefore be indicated by Fig. 20.

Fig. 20



Thigmotactic Paramœcium, showing direction of water currents with electric circuit *open*.

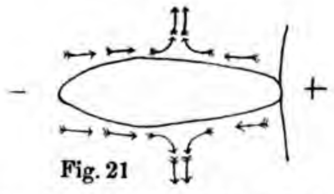


Fig. 21

Same individual shown in Fig. 20, after closing the electric circuit.

If now the electric circuit be closed, with cathode and anode in the position shown, the current of particles is quickly *reversed* on the posterior half of the animal, but continues in the same direction on the anterior half. The result is shown in Fig. 21.

If now the electric current is reversed, so that the cathode and anode change places, the direction of the stream of granules over the posterior half of the body is likewise reversed, passing again to the rear as at the beginning. The animal retains its position undisturbed, however; or sometimes it swings into an oblique position, so that the effects are no longer clear. But often the reversal may be repeated a large number of times, the animal remaining in place. I will copy from my record two typical cases of this sort.

(*a*) Paramœcium as shown in fig. 20. With the electric circuit *open* the stream of particles is to the rear. Circuit closed with the posterior end to the cathode; the stream of particles now passes *forward* on the posterior half of the animal. Electric current reversed; stream of particles likewise reversed, passing again to the rear. In 30 seconds the electric current was reversed 13 times, accompanied in every case by a reversal of the stream of granules. The circuit was then opened and the animal was observed 45 seconds longer; no reversals occurred during this time.

(*b*) Paramœcium as in fig. 20. Electric current reversed 10 times in 20 seconds, accompanied each time by a reversal of the stream of granules. Then the circuit was open for 30 seconds; no reversal during this time.

If however instead of reversing the electric current we allow the circuit to remain closed, with anode and cathode as shown in Fig. 21, we find that the current of granules over the posterior half of the animal *does not continue* to pass forward, as in Fig. 21, but in a few seconds the currents begin to flow again in their original direction (Fig. 20). The electric circuit remaining continuously closed, in a few seconds there is another reversal of the current at the posterior end (Fig. 21), lasting but an instant, then a return to the original direction. Then another reversal; another return, and so it continues indefinitely. A few extracts from my notes, records of actual cases observed, will bring out the conditions and limitations of this phenomenon. The position of the animal was in every case as shown in Fig. 20. A "reversal" signifies a period when the cilia strike *forward* over the posterior half of the body.

(*c*) The animal was watched 40 seconds continuously, with *open* circuit; during this time the stream of granules was always to the rear. Circuit closed; current of particles at the posterior end reversed, then returned to the original direction. Circuit remained closed 25 seconds, during which time there were seven such reversals, at intervals of 2 to 5 seconds. The reversals became more frequent in the latter part of the period; the animal became restive, swung back and forth, till finally it swung entirely out of its position and was not observed farther.

(*d*) Six reversals of the current of granules in 15 seconds with closed circuit.

(*e*) With circuit *open*, no reversals in 45 seconds. Circuit closed; three reversals in 60 seconds.

(*f*) Circuit closed; six reversals in 18 seconds; circuit opened, no reversals in 12 seconds.

(*g*) Circuit closed for 60 seconds; nine reversals at nearly equal intervals. Each reversal commonly lasted but an instant; sometimes however 4 or 5 seconds.

(*h*) Circuit closed 30 seconds; 10 reversals.

(*i*) Circuit closed; no reversals for 15 seconds; then six at intervals of about three seconds; then no more for 30 seconds. Observations interrupted.

(*j*) Circuit open for 30 seconds; no reversals. Closed 90 seconds; 8 reversals. Circuit continues closed 60 seconds longer, but no more reversals occur.

(*k*) Circuit closed 28 seconds; 18 reversals in this period.

From the above it appears—

(1) That the current of granules, originally toward the posterior end in thigmotactic individuals, is reversed at the cathode end when the animal is subjected to the electric current with the cathode at the posterior end;

(2) That the reversal does not last, but the current produced by the cilia quickly returns to its original direction, *even when the electric circuit remains closed.*

(3) That the length of time which the reversal lasts varies from an instant to several seconds.

(4) That the interval of time between the reversals varies from a fraction of a second (case *k*) to 20 or more seconds (case *e*).

(5) That in some cases the electric circuit may remain closed a considerable time without causing any reversals (cases *i* and *j*).

(6) That sometimes the first reversal does not occur till some seconds after the circuit is closed (case *i*). This is often true.

I may add—

(7) That cases occur, though rarely, where the ordinary current used (10 cells) causes no reversals at all. In other cases, while no actual *reversals* of the currents of particles occur, sudden and repeated *stoppages* indicate the effect of the electric stimulus.

In Paramœcia occupying the positions shown in Fig. 20 no change was observed in the currents of granules over the anterior half of the animal when the electric circuit was closed.

If the Paramœcium lies in a position the reverse of that shown in Fig. 20—that is, if its anterior end is to the cathode, the posterior to the anode—the effect of the electric current on the stream of granules is not so noticeable as in the foregoing case. If the stream of granules over the posterior half of the animal (anode end) is already toward the rear when the circuit is open, closing the circuit simply increases the strength and rapidity of the current. In the few cases where the current of granules is obliquely forward at the posterior end of the animal when the circuit is open, closing the circuit with the anode at the posterior end causes a reversal of this current—the granules passing to the rear. This case—the posterior end of the animal to the anode—is much more difficult to study than the opposite one, because the animals generally swing quickly into another position when the circuit is closed. At the anterior end (toward the cathode) commonly no change is visible. In a few cases, by direct observation of the cilia, they seemed to take a position such as to be directed a little more *forward*, as would be expected from Ludloff's results. In other cases however precisely the *reverse* change seemed to take place. But it is only natural that the results here should not be clear, when one considers the effect of the motion of the cilia over the posterior half of the body. The strong backward current of particles in this region produced by the closing of the circuit corresponds of course to a strong tendency to push the animal itself forward. As the anterior end is generally partly imbedded in the soft mass of decaying plant tissue, the forward push from behind tends naturally to bend the anterior cilia backward. Hence the variation in the observation, due to the varying ratio of the effects produced by the current itself (inclining the cilia forward, according to Ludloff) and the push from behind (inclining the cilia backward). The effect of the electric current on the posterior half of the body is clear; on the anterior half (by this method of study) it is not.

The third case which calls for discussion is that in which the animal lies transversely to the direction of the current. Suppose the current of particles is everywhere to the rear when the circuit is open. The circuit is now closed with anode and cathode on opposite sides of the animal. Immediately there is a reversal of the stream of granules on the cathode side, the granules here passing *forward*, while on the anode side it continues backward as before. The currents under this condition are shown in Fig. 22. If now the electric current is reversed, the streams of granules on both sides of the animal are reversed—passing backward on the previously cathode side (now turned toward the anode), and forward on the previously anode side (now turned to the cathode). The change in the direction of the motion of the cilia naturally produces some change in the position of the body of the infusorian, the animal swinging on its fixed anterior end as a pivot a certain distance towards the cathode. Therefore as the electric current is successively reversed, the animal oscillates back and forth.

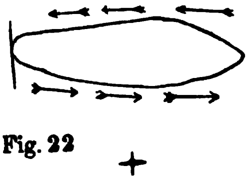


Fig. 22

Some examples may be given of the phenomena described.

(*l*) Paramœcium with the left side to the cathode; stream of particles on both sides to the rear when the circuit is open. Electric circuit closed; stream on left side now forward. Circuit open; stream on both sides again to the rear. Circuit closed three times in succession, each time producing a reversal on the cathode side.

(*m*) Animal transverse to the electric current, with the stream of granules on both sides to the rear. Circuit (30 cells) closed; stream of granules reversed on the cathode side, passing *forward*, and the animal swings toward the cathode. Electric current reversed; stream of granules on both sides reversed, and the animal swings in the opposite direction—toward the new cathode. Reversal repeated 25 times, always with the same result. The animal does not leave its place.

If the circuit remains closed after having caused a reversal of the at first backward stream of granules on the cathode side, in a few seconds the stream of granules returns to its original direction. The circuit remaining closed, the stream of particles is again reversed on the cathode side after a short interval. These oscillations continue, exactly as described in detail for the case in which the posterior end is directed to the cathode,—except that in the transverse position it is only one side which shows the repeated reversals—the stream of granules remaining always directed to the rear on the anode side.

In interpreting these results we must keep in mind the normal motion of the cilia in thigmotactic individuals. As described previously, in such animals the oral cilia strike strongly backward, while the locomotor cilia show merely an ineffective quivering. The reversal of the current on the cathode half of the body indicates thus that the locomotor cilia of this half are suddenly set in motion, such that they strike forward. It does not imply however a reversal of a previous motion of these cilia. The conflict between the two stimuli is therefore to be expressed as follows. The contact stimulus tends to keep the locomotor cilia at rest; the electric stimulus to set them into strong motion—directed forward at the cathode side or end; backward at the anode side or end. Whether the electric stimulus has an effect on the oral cilia also is not determined by these observations.

In summing up, we may say:

1. (*a*) On the anode end or side the effect of the constant electric current is to drive the stream of particles to the rear. The cilia are so affected as to make the backward stroke stronger.

(*b*) On the cathode end or side the effect of the constant electric current is to drive the stream of granules forward. The cilia are so affected that the forward stroke is made stronger.

For the anterior end these phenomena were not determinable by the method used, but the results were clear for the other parts of the body.

The results are thus (omitting from consideration the undetermined point just mentioned) a confirmation of Ludloff's results. I have thought it worth while to emphasize them, as they were gained by a different method from that used by Ludloff.

2. A *struggle* takes place between the contact stimulus and the stimulus of the constant electric current. The characteristic arrangement of the locomotor cilia in thigmotaxis is overcome by the electric current for an instant, then the contact stimulus resumes its sway; then the electric; and thus the oscillations continue. The two stimuli do not unite and give a resultant reaction; the cilia seem to react at a given time to one or the other alone. With the ordinary electric current that suffices easily to direct the free swimming Paramœcia to the cathode, the contact stimulus shows itself the stronger and the infusoria do not swim to the cathode, but remain in contact with the solid body. With a very strong current (30 small chromic acid cells newly filled), the action of the electric current is very powerful; the reversals in direction of the water currents and the accompanying changes in position of the Paramœcia become convulsive in character. One by one the Paramœcia loosen from their position and swim to the cathode. There are very great individual differences in regard to this; some few individuals retained their position on the bit of filter paper in spite of the strongest current I could produce with the battery at my disposal. But the great majority may be forced by such a current to finally leave the solid body and swim to the cathode.

I do not feel prepared at present to enter upon a discussion of the significance of this struggle between two stimuli. Probably the phenomena here described may find explanation from some simple point of view when the precise nature of reactions to stimuli in unicellular organisms is better understood, and especially if the essential nature of the effects of the electric stimulus become better known.

4. PART PLAYED BY THESE REACTIONS IN THE NORMAL LIFE OF PARAMŒCIUM.

Examination of the distribution and conduct of the Paramœcia in an ordinary culture jar indicates that three stimuli play the chief *rôles* in the daily activities of these infusoria. These are (1) negative geotaxis, (2) positive thigmotaxis, (3) positive chemotaxis toward CO_2.

The effect of *negative geotaxis* is at once evident in the presence of all the Paramœcia in the upper part of the water. The geotaxis of Paramœcium has been minutely studied by Jensen, so that no discussion is necessary here.

That *thigmotaxis* plays an important part in the daily life of the infusoria is shown by the fact that the large majority of them will generally be found clinging to some solid body. A large number are attached to the decaying plant tissue just below the surface of the water. As we have seen, there is no evidence that this material in itself exercises a chemical attraction for the Paramœcia, and they gather in exactly the same manner on entirely neutral substances. A second very large portion of the infusoria are gathered in a narrow band on the glass wall of the jar, not far below the upper surface of the water; here of course an active chemical action of the substratum is out of the question. The action of the cilia in thigmotactic individuals is, as we have seen, such as to tend to bring food into the mouth.

The *rôle* played by positive chemotaxis towards CO_2 in the conduct of Paramœcia under various circumstances has already been elucidated in a considerable number of examples (p. 290). In the culture jars the effect of this reaction is seen in the collecting together of the infusoria into dense assemblages. For example, the thigmotactic Paramœcia above described are not scattered here and there over the surface of the glass, but are gathered in a close band, not far below the upper surface of the water. The exact level at which this assemblage takes place is determined chiefly by the rapidity of the escape of CO_2 from the water surface. The Paramœcia tend of course to rise as high as possible, owing to their negative geotaxis. But close to the surface the carbon dioxide escapes so fast that the Paramœcia sink lower and remain at a level where the CO_2 produced by themselves reaches a concentration to which they are attracted. The same is true of the infusoria gathered on the decaying plant tissue. Moreover, even at the level where the gathering is formed, the Paramœcia are not collected in all parts in like numbers. Here and there dense assemblages, where the animals are gathered literally into masses, are found; these are due to the crowding together of the Paramœcia, owing to their positive chemotaxis toward the CO_2 produced in such a gathering.

Other reactions seem to play a less important part in the life activities of Paramœcium. *Thermotaxis* is sometimes noticed in the collecting of the animals on the warmer side of the jar; in my cultures however this reaction seemed to come rather rarely into play. It is probable that in the case of Paramœcia subjected to great variations in temperature, as may sometimes occur under natural condition, thermotaxis plays an important *rôle*. *Electrotaxis* is of course unknown in the normal life of Paramœcium. Positive chemotaxis toward other substances than carbon dioxide seems to have no important place in the normal life activities of the animal, and negative chemotaxis is scarcely to be observed under natural conditions. The three reactions first discussed seem to be the chief determining factors in the life activities of Paramœcium.

5. General Discussion.

The present paper is to be considered as merely the first of a series the purpose of which is to contribute to the knowledge of the essential nature of reactions to stimuli in single-celled organisms, and especially to investigate the relations of the phenomena studied to morphogenesis. It is hoped that at the end of the investigation thus begun some general conclusions of value may be presented, but it does not enter into the plan of this first contribution to discuss at length the broad general problems which this field of work offers. Certain phenomena above described which have a definite bearing upon some of these general problems may however here be emphasized.

All the reactions studied in the foregoing investigation are expressed by some change in the motions of the cilia. Before therefore conclusions as to the nature of these reactions can be gained, the exact changes in the motion of the cilia, and the precise relation of these changes to the stimulus causing them must be determined. For Paramœcium the only reaction which may be said to have been studied in this manner is electrotaxis, as presented in the work of Ludloff. A similar method must be followed for other stimuli before satisfactory general conclusions can be gained. An investigation carried out on these lines would have such questions as the following to answer:— How do the cilia react to localized stimuli? For example, does the same stimulus produce the same result whether applied to the anterior end, the posterior end, or the sides of the animal? Are these characteristic reactions for the different sorts of stimuli—as for different chemical reagents, and what are these reactions? If the stimulus is continued, do the reactions change? What differences are there between reactions to sudden and to gradual stimuli? Is there any single characteristic reaction to *all* very strong stimuli?

For determinations of these questions three methods may be used; observation of the motion of the animal's body, observation of the currents caused by the motion of the cilia, and direct observation of the motion of the cilia, under the influence of the proper stimuli. All these methods together would doubtless be necessary for accurate results in all points. In the foregoing investigation only the two first were used to any extent, and of course no attempt was made to answer all the above questions, yet some results were gained which would take their place in a systematic investigation of the sort above suggested.

For the contact stimulus, a characteristic position and motion of the cilia was demonstrated. According to the results gained in this field, we must distinguish in Paramœcium two different systems of cilia, whose reactions must be studied separately: these are the *oral* cilia, which lie in the oral groove, and the *locomotor* cilia, covering the rest of the body. The oral cilia were shown to have in thigmotaxis a definite method of action, resulting in well-characterized currents of water or motions of the animal's body. In the case of the locomotor cilia it is necessary to distinguish those which are in direct contact with the solid body, and those which are free. The former are held stiff, straight and immobile; the latter keep up an ineffective quivering. The ciliary motion when the animal was acted upon at the same time by a contact stimulus and the electric stimulus was also studied, and shown to consist in an alternate prevalence of the reaction due to each of the two stimuli taken separately. The two stimuli do not combine to produce a reaction which is the resultant of the two influences, but they alternate in controlling the motion of the locomotor cilia. The observations on the reaction to the contact stimulus show the exceedingly complex character of reactions, even in a unicellular organism. A single stimulus produces three different results in three different regions of the body. A renewed investigation both of electrotaxis alone and of its combination with thigmotaxis, with relation to the precise action of the *oral* cilia, as distinguished from the others, might bring out some new relations of value for our analysis. The division of the body of the infusorian into two ends and an intermediate surface or "sides" is evidently entirely inadequate for an exact analysis of the reactions of the cilia; it is quite possible that other regions beside the oral groove will be found to take a separate position.

For chemical stimuli definite results were gained on certain of the questions above proposed. We will here leave out of consideration the question whether the Paramœcia gather about a source of stimulus or flee from it; such activities are too complex to give precise results as to the motion of the cilia. We will discuss here only such activities as indicate exactly the motion of the cilia causing them.

When the anterior end of an animal swimming forward comes in contact with a strong chemical stimulus, the reaction takes the form of a *reversal* of the effective motion of the cilia; the *forward* stroke becomes strongest, driving the animal *backward*. The strength and duration of the reaction bear a clear relation to the strength and quality of the stimulus. A weak stimulus causes only such a reversal as drives the animal backward but a fraction of its own length, while if the infusorian comes in contact in the same manner with a drop of strong acid it darts backward a long distance—twenty times its own length or more. It appears that this form of reaction is not characteristic for any particular kind of stimulus; a similar (though weak) darting back is observed when the infusorian strikes a mechanical hindrance. It is probable that any strong stimulus acting upon the anterior end of the animal causes the cilia to strike suddenly forward, thus forcing the animal backward.

The same effect is often produced if a sudden strong stimulus acts upon the animal from all directions at once,—that is, if the stimulus is not localized at all. Thus if the Paramœcia are suddenly dropped into a solution of some chemical which affects them strongly but does not at once kill them they begin at once to swim backward. The same is true if they are introduced suddenly into a chemically indifferent solution having a strong osmotic action, as into a solution of sugar or glycerine. Sudden introduction into water heated to 34° C. has the same effect.

For studying the nature of reactions due to a stimulus acting upon some definitely localized region of the body, other than the anterior end, other methods will have to be used, and the investigation will doubtless be accompanied with great difficulties. For the case of a stimulus acting upon one side, from which the Paramœcia turn away, as for alkalies and many other substances, three possible reactions suggest themselves. (1) The cilia on the side touched may have their effective backward stroke increased in power. This would turn the animal away from the source of stimulus, but would at the same time urge the animal slightly forward. (2) The cilia on the side opposite the source of stimulus may have their effective stroke reversed, so as to strike most strongly forward. This would turn the animal away from the source of stimulus, and tend at the same time to drive it backward; it would therefore be apparently a more advantageous form of reaction than the foregoing, in the case of a strong and injurious stimulus. (3) There may be some combination of these two methods of reaction.

Similar considerations apply to the case of a one-sided stimulus to which the animals are positive. After these problems have been thoroughly worked out it will be possible to compare them with the reaction to the electric current described by Ludloff, and thus to gain some basis for a judgment in regard to a possible relation of the electric stimulus to the chemical effects of the constant current,—such as is suggested by Loeb and Budget.

Passing to a slightly different aspect of the subject, a peculiarity of the manner of reaction of Paramœcium to chemical substances may here be emphasized. The animal seems to respond only when there is a distinct and sudden change in the chemical nature of the medium. If a drop of some attractive substance is introduced into the centre of a preparation containing Paramœcia, they do not swim in straight radial lines to the drop as a centre. On the contrary, the course of the individual is apparently not modified in the least until an animal strikes in its random course the margin of the diffusing drop; then the characteristic reaction takes place. Furthermore, the collection of Paramœcia in a region containing an attractive substance gives the impression of being due rather to a negative taxis to the surrounding fluid, after having entered the attractive solution. The Paramœcium often swims into the drop of attractive substance without showing any indication of stimulation, but as it attempts to pass out on the other side a strong negative stimulus occurs; the cilia are wholly or partly reversed, and the animal remains in the drop.

Turning now to questions of a different character, we may ask what is the bearing of the fact that Paramœcium is negatively chemotactic to the water in which it lives? As I have shown, the water containing decaying vegetable material, in which the infusoria live and thrive, has an alkaline reaction, and the general rule that Paramœcia are negatively chemotactic to alkaline fluids suffers no exception in this case. The negative chemotaxis toward this fluid is shown by the avidity with which Paramœcia immersed in it gather into a drop of neutral fluid, such as distilled water or sugar solution, as well as by the fact that Paramœcia already in a neutral solution avoid without exception a drop of water from the culture jar. What relation has this to the subject of *acclimatization* or adaptation to the environment?

It seems to me that we are driven to the simple conclusion that negative or positive taxis is not a test of adaptation. It cannot be doubted that the Paramœcia are thoroughly acclimatized to the solution in which they live. Here they thrive and multiply immensely. Yet they are strongly negatively chemotactic to this same solution.

A related problem is presented by the strong positive chemotaxis of Paramœcium toward carbon dioxide. This reaction plays a most important part in the life of the organism; in fact I believe that it is not too much to say that it is the chief directive influence affecting Paramœcium. Yet we know that carbon dioxide is decidedly injurious to most animal protoplasm, and Loeb and Hardesty have shown that it is a positive poison for this same infusorian Paramœcium,—acting in virtue of its own chemical properties and not merely through its taking the place of the necessary oxygen.

Doubtless other protozoa will be found that are attracted by CO_2. This is rendered especially probable for certain infusoria by the observations of Pfeffer, already referred to. Pfeffer (p. 618) observed such collections of individuals as are described in this paper for Paramœcium, in the case of *Glaucoma scintillans*, *Colpidium colpoda*, *Stylonychia mytilus*, and in Paramœcium itself. As I have tried to show on p. 294, the collections in all these cases are probably due to the same cause as in Paramœcium—positive chemotaxis toward the excreted carbon dioxide.

We must not forget however that Paramœcium is repelled by a concentration of CO_2 that is strong enough to be injurious. This would seem to make it probable that such solutions of CO_2 as are attractive, are so because they are beneficial. That carbon dioxide should serve any beneficial purpose in the animal cell seems however on general grounds highly improbable, and this improbability is increased by the fact that the CO_2 by which the Paramœcia are attracted is excreted by the Paramœcia themselves. It seems exceedingly paradoxical that an organism should excrete a substance to which it is strongly attracted. Yet this is undoubtedly the case. The paradox seems almost to rise to an absurdity however if it is held that the animals are attracted to this excreted substance because they *need* it; in that case why should it have been excreted?

It is perhaps comprehensible that an organism should sometimes be attracted by an injurious substance with which it normally never comes in contact. One might hold in such a case that the reaction toward this substance has never been determined by natural selection or otherwise; the organism might therefore react to this in the same way as toward some other substance having a somewhat similar action, but uninjurious, with which the animal *has* previously come into relation. Such a reaction might perhaps be called a mistake on the part of the organism, due to the similarity of two substances, one beneficial, the other injurious. In other words, the protoplasm has never become so differentiated as to give a different reaction with these two substances. It is of course a well-known fact, brought out in Pfeffer's researches, that organisms *are* often attracted by substances with which in a state of nature they never come in contact, these substances having a chemical similarity to certain others which form a customary means of attraction. Thus, it is known that potassium chloride is strongly attractive to certain bacteria and doubtless plays an important part in their life activities. These same bacteria may be likewise attracted by the closely related substance rubidium chloride, though under normal circumstances they undoubtedly never come in contact with this.

But any such explanation is of course entirely excluded in the case of carbon dioxide—a substance with which the Paramœcia are continuously in contact, in one way or another, especially as the reaction due to it plays such an important part in the life activities of the animal. The possibility occurs to one that the positive chemotaxis toward CO_2 may have some relation to the organisms upon which Paramœcium feeds—the bacteria of decaying vegetable material. There seems however to be nothing known to suggest this possibility. The evident result of the positive chemotaxis of Paramœcium toward CO_2 is the gathering together of the infusoria into dense collections, but what purpose is served by these collections does not appear.

The attraction of Paramœcia toward self-excreted CO_2 seems to throw light on a general question proposed by Pfeffer; namely, whether it is necessary, in order that a substance should act as an attractive stimulus, that it should be taken into the organism. As the CO_2 in the water was first produced within the Paramœcia, and acts as a stimulus after it has been given off to the outside, it appears that the stimulus must be an external one; Pfeffer's question, in accordance with this, is to be answered in the negative.

6. SUMMARY.

1. Paramœcium aurelia is strongly positively chemotactic to a solution of *carbon dioxide* in water.

Carbon dioxide is excreted by the Paramœcia in quantities sufficient to be detected with proper reagents. By this excreted CO_2 the infusoria are attracted; hence they tend to gather into dense groups. This positive chemotaxis to the carbon dioxide excreted by themselves plays a very large part in the normal activities of Paramœcia.

2. Paramœcium is also positively chemotactic toward all weak acids and all solutions having an acid reaction, so far as tested.

Toward *strong* solutions of all acids, including CO_2, the infusoria are *negatively* chemotactic.

3. Paramœcium aurelia is *negatively* chemotactic to the fluid (water containing decaying vegetable matter) in which it lives and thrives. This fluid has an *alkaline* reaction. Paramœcium is likewise negatively chemotactic, so far as tested, to *all* solutions having an alkaline reaction. It is also negatively chemotactic to certain neutral salts and organic compounds.

4. Toward certain organic substances, as sugars, glycerine, urea, Paramœcium is entirely *indifferent*, so far as chemical properties are concerned.

5. Paramœcia which are collected in a drop of a solution to which they are positively chemotactic, show a modified reaction to the constant electric current. They swim toward the cathode till the boundary of the drop of solution to which they are positively chemotactic is reached, but cannot be forced past this boundary into the surrounding water, except by a very powerful and long-continued electric current.

6. *Tonotaxis*, the directive stimulus due to a change of osmotic pressure, acts only within wide limits, so that for most solutions it does not come into action at all, the chemical qualities of the solution determining the conduct of the Paramœcia long before the limit at which tonotaxis becomes effective is reached. While great variation in osmotic pressure does act as a stimulus, this stimulus is not of sufficient strength and definiteness to prevent the infusoria from swimming into solutions of such osmotic power that they are quickly killed. Tonotaxis therefore plays no important part in the life activities of Paramœcium.

7. Paramœcium responds in a characteristic way to the stimulus of

contact with solid bodies. This reaction consists of the following factors; (1) the Paramœcium places itself against the solid body (positive thigmotaxis), the cilia which are in actual contact with it being held straight, stiff and immovable; (2) the oral cilia have a strong and characteristic motion; (3) the locomotor cilia are nearly at rest, merely quivering ineffectively.

8. If through a preparation containing Paramœcia exhibiting the thigmotactic reaction a constant electric current is passed, the Paramœcia do not swim to the cathode, as under other circumstances, but remain in contact with the solid body.

Exact observation shows that under such circumstances the two stimuli alternate in controlling the locomotor cilia. On the cathode side or end the cilia are so affected by the electric current that the effective stroke is forward (as described by Ludloff), thus driving the water currents in the opposite direction from that which they have under the thigmotactic stimulus alone. But this reversal lasts but an instant; then the thigmotactic stimulus resumes its sway and the water currents again pass backward. The electric circuit remaining closed, another reversal takes place in a few seconds, then another return to the original direction, and this alternation of reactions may be repeated many times.

9. The reactions which play the chief part in the normal life of Paramœcium are *negative geotaxis*, *positive thigmotaxis*, and *positive chemotaxis toward carbon dioxide.*

The foregoing work was done in the Physiological Institute of the University of Jena, during the winter semester of 1896–97. It gives me pleasure to acknowledge here my great obligation to Prof. M. Verworn for much valued counsel and assistance throughout the work. I wish to express my thanks also to the Director of the Institute, Prof. Biedermann, for placing at my disposal in the most obliging manner the space and resources of the laboratory.

JENA, *March* 2, 1897.

LITERATURE CITED.

1896. **Bokomy, Th.** Archiv f. d. ges. Physiol., LXIV. pp. 262—306.

1886. **Dewitz, J.** Pflüger's Archiv f. d. ges. Physiol., XXXVIII. pp. 358—385.

1894. **Greenwood, M., and Saunders, E. R.** This Journal, XVI. pp. 441—467.

1886. **Hamburger, H. J.** Archiv f. Anat. u. Physiol. (Physiol. Abth.), Jahrg. 1886, pp. 476—487.

1897. **Israel, O. und Klingmann, Th.** Archiv f. pathol. Anat. CXLVII. pp. 293—340.

1893. **Jensen, P.** Archiv f. d. ges. Physiol. LIII. pp. 428—480.

1890. **Loeb, J.** Der Heliotropismus der Thiere und seine Uebereinstimmung mit dem Heliotropismus der Pflanzen. Würzburg. 118 pp.

1896. **Loeb, J.** Archiv f. d. ges. Physiol. LXV. pp. 308—316.

1897. **Loeb, J. und Budget, S.** Archiv f. d. ges. Physiol. LXV. pp. 518—534.

1895. **Loeb, J. und Hardesty, I.** Archiv f. d. ges. Physiol. LXI. pp. 583—594.

1895. **Ludloff, K.** Archiv f. d. ges. Physiol. LIX. pp. 525—554.

1888. **Massart, J.** Bull. Acad. Roy. de Belgique, 3^e série, XV. No. 5.

1889. **Massart, J.** Archives de Biologie, IX. pp. 515—570.

1889 (*a*). **Massart, J.** Bull. Acad. Roy. de Belgique, 3^e série, XVIII. pp. 215—220.

1895. **Mendelssohn, M.** Archiv f. d. ges. Physiol. LX. pp. 1—27.

1888. **Pfeffer, W.** Untersuchungen a. d. bot. Inst. z. Tübingen, II. pp. 582—661.

1890. **Schürmayer, C. B.** Jenaische Zeitschr. XXIV. pp. 402—470.

1889. **Verworn, M.** Psycho-physiologische Protisten-Studien, Jena, 218 pp., 7 Tafeln, 27 Textabbildungen.

1889 (*a*). **Verworn, M.** Archiv f. d. ges. Physiol. XLV. pp. 1—36.

1890. **Verworn, M.** Archiv f. d. ges. Physiol. XLVI. pp. 267—303.

1896. **Verworn, M.** Archiv f. d. ges. Physiol. LXII. pp. 415—450.

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STUDIES ON REACTIONS TO STIMULI IN UNICELLULAR ORGANISMS. II. — THE MECHANISM OF THE MOTOR REACTIONS OF PARAMECIUM.

BY HERBERT S. JENNINGS.

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STUDIES ON REACTIONS TO STIMULI IN UNICELLULAR ORGANISMS. II. — THE MECHANISM OF THE MOTOR REACTIONS OF PARAMECIUM.[1]

BY HERBERT S. JENNINGS.

AS pointed out in No. I. of these Studies,[2] most of the reactions of Paramecium (as well as other Protozoa) are known only in their general features. The mechanism of the reactions, — that is, the motions of the cilia by which they are brought about, has been determined for Paramecium only in Ludloff's study[3] of electrotaxis and my own of thigmotaxis. It is the purpose of the present paper to supply the lack thus indicated by giving a detailed account of the mechanism of the motor reactions of Paramecium.

The far-reaching importance of an accurate knowledge of the exact mechanism of the reactions of Paramecium is apparent when we recall the sweeping conclusions that have been drawn from the results of studies on the reactions of this and other unicellular organisms. Theories of the mechanism of thermotaxis, geotaxis, and other reactions have been put forth, accompanied by deductions as to the remarkable sensitiveness of protoplasm to the various classes of reagents, and these theories have borne an important part in forming various prevalent conceptions in general physiology and in comparative psychology. They depend for their validity on whether the mechanism assumed for the reactions is the correct one, and for this assumption a basis of observation has usually been lacking. As will appear, some conclusions that have been deduced with the utmost confidence lose their validity when the actual mechanism of the reactions becomes clear.

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[1] SCIENTIFIC RESULTS OF A BIOLOGICAL SURVEY OF THE GREAT LAKES, directed by Jacob Reighard, under the auspices of the U. S. Fish Commission, No. 1. (Published by permission of the Hon. George M. Bowers, Commissioner of Fisheries.)

[2] JENNINGS, H. S.: Journal of physiology, xxi, pp. 258-322.

[3] LUDLOFF: Archiv f. d. ges. Physiol., 1895, lix, p. 525.

It has become the fashion to refer many processes taking place in ontogeny to chemotaxis, or other reactions to stimuli, the matter apparently being considered as therewith definitely put at rest, as if such reference permitted no further analysis. It seems often to be forgotten that movements induced by chemotaxis must be accomplished by some means, some mechanism; and upon the character of this mechanism depends largely the interpretation to be given to the process. It may aid in recalling this neglected fact to set forth the roundabout method by which certain free cells succeed in gathering about a source of attractive stimulus.

A third standpoint, from which a knowledge of the general mechanism of reactions to stimuli should be of interest, is that of comparative psychology. The stage of mental development represented by unicellular organisms has, since the work of Verworn, been studied comparatively little, at least in any thorough manner, and the disconnected phenomena observed have lent themselves to interpretation by different authors in the most varied ways. Some hold that such organisms have a complex psychology containing nearly all the elements of which the psychic life of higher animals is made up, while others maintain that the observed phenomena are explicable on the simplest grounds; that such organisms are merely automata of the most limited capabilities, and that the activities which they show require little more than the property of irritability for their explanation. It may be hoped that an exact knowledge of the mechanism of the reactions of one of these organisms may throw further light on the question of the simplicity or complexity of the "psychic life of micro-organisms."

With these problems in mind, an examination will be made of the motor reactions of Paramecium. The organism to be studied, Paramecium caudatum,[1] is a unicellular animal belonging to the group of ciliate infusoria, and is well known in every biological laboratory, living by thousands in vegetable matter decaying in water. It is somewhat cigar-shaped in form, having a narrow but blunt anterior end and a broad, sharp posterior end. From the anterior end a broad depression known as the oral groove runs obliquely along one side (the oral side) to the mouth, in the middle of the body. Near the opposite side (the aboral side) are the two contractile vacuoles. The entire surface of the body is covered with cilia, by means of which the animal moves. The length of the animal is about 0.2 mm.

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[1] In the first of these Studies (*loc. cit.*) the organism used for experimentation is called Paramecium aurelia. I adopted this name because the organism has been always so called in the Jena Physiological Institute, where the work was done, and because my attention had not been called to the possibility of a mistake

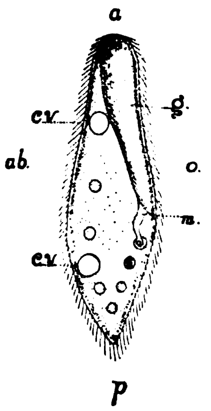

FIGURE 1. Paramecium caudatum. *a*, anterior end; *p*, posterior end; *ab*, aboral side; *o*, oral side; *m*, mouth; *g*, oral groove; *cv*, contractile vacuoles.

During its progress through the water, Paramecium comes in contact with sources of stimuli of various kinds; it strikes against mechanical obstructions, compelling it to turn aside, or it meets with various chemical substances in solution which either attract or repel it: heat, electricity, and other agents may likewise act as stimuli; to all these the animal reacts in a characteristic manner. The problem for solution is, exactly *how* is such a reaction accomplished? How does the attractive chemical compound succeed in affecting the locomotor organs of Paramecium so that it turns toward the source of stimulus? How does the repellent compound succeed in turning the Paramecium away from the source of stimulus?

In the first of these "Studies on Reactions to Stimuli" details were given as to the general features of the phenomena shown in the reactions of Paramecium. It was there shown that Paramecia are positively chemotactic to weak solutions of substances having an acid reaction, negatively chemotactic to substances having an alkaline reaction, and to many neutral salts. I shall give here in brief only enough of the gross appearance of these reactions to make intelligible the account of the finer mechanism and the way in which this mechanism is demonstrated.

in the name. But it appears that systematists make a distinction between Paramecium aurelia, with two micro-nuclei, and caudatum, with but one. As the Paramecia which I have been studying at Hanover, N. H., have but one micro-nucleus, I must call them P. caudatum. I believe them to be identical with the organisms used at Jena, though I did not investigate the micro-nucleus there.

The method by which the reactions of Paramecium toward most stimuli is demonstrated in practice is shown in Fig. 2. Paramecia are brought upon a glass slide and covered with a long cover-glass, supported near its two ends by bits of capillary glass tubing. The Paramecia swim at random throughout the preparation. Now a drop of any solution toward which the method of reaction is to be tested is introduced beneath the cover-glass by means of a capillary pipette, as shown in the figure. The Paramecia in their random swimming strike the edge of the drop, whereupon they react in a characteristic manner. If they are positively chemotactic to the substance in the drop — as they are if it is weakly acid in reaction — they soon gather in a dense swarm within the drop, as shown in Fig. 3. If, on the other hand, they are negatively chemotactic, the drop remains entirely empty, as shown in Fig. 4. This method is of course designed especially for the demonstration of chemotaxis. Thermotaxis, or the reaction toward heat or cold, may be demonstrated in a similar manner, or better by warming or cooling one end of the slide; still better by means of the apparatus devised by Mendelssohn.[1]

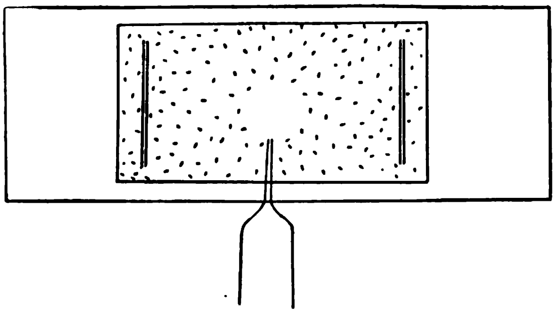

FIGURE 2. Method of testing the reactions of Paramecium.

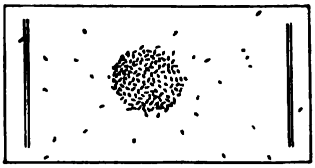

FIGURE 3. Paramecia collected in a dense swarm in a drop of a solution to which they are positively chemotactic.

Now, how does the substance in the drop in Fig. 3 succeed in affecting the cilia in such a way as to make the Paramecia turn toward and enter the drop?

Observation of the method by which the Paramecia collect in the drop shows that the foregoing question involves an assumption which is untrue. The Paramecia in the neighborhood of the drop do not turn toward it. The animals collect in the drop in an entirely different way. Suppose the drop to have been just introduced, as in Fig. 2. A Paramecium in its random course strikes by chance against the margin. It does not react, but keeps on its way across the drop until it comes to the other side (Fig. 5). There it reacts negatively, and turns back, swimming across the drop in another direction, till it again comes to the margin of the drop. There it again reacts negatively, and swims in a new direction, till it is turned back by the margin as before. This continues, so that the Paramecium is, as it were, imprisoned in the drop. Fig. 5 shows the course of a single Paramecium in such a drop. Other Paramecia enter the drop in the same way, purely by chance, and remain in the same manner, until the drop swarms with Paramecia. Owing to the restless hither and thither swimming of the animals on the slide, almost every one will in a short time have come by chance against the edge of the drop, will have entered and remained. Thus in a short time we get the appearance shown in Fig. 3; almost all the Paramecia in the preparation are collected in the drop. We say that the Paramecia are positively chemotactive to the substance in the drop, but so far as any motor reaction is concerned, evidently a more accurate statement is that after entering the drop by chance they are negatively chemotactic to the surrounding fluid.

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[1] MENDELSSOHN: Archiv f. d. ges. Physiol, 1895, lx, p. 1.

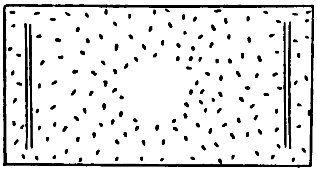

FIGURE 4. Negative chemotaxis toward a drop of sodic carbonate solution introduced 10 minutes before.

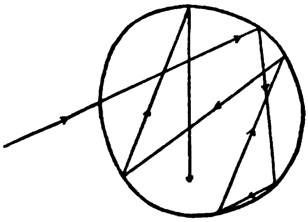

FIGURE 5. Course of a Paramecium in a drop of attractive substance.

The same thing may be shown to be true for thermotaxis or the reaction toward heat. In place of the chemical solution, we may introduce into a slide preparation of Paramecia in cold water a drop of water warmed to the temperature toward which the Paramecia appear to be positively thermotactic. We shall find that the Paramecia collect in the warm drop in exactly the same manner as in the case of the chemical compound just described: they enter by chance, then are negatively tactic to the surrounding cold water.

An extended examination of the reactions of Paramecium shows that this is typical for all apparently positive reactions. *In and of* *itself, considered as a motor reaction, there is no positive taxis in Paramecium.* The so-called positive taxis always acts indirectly through (apparent) negative taxis. The question of how Paramecium turns toward an attractive substance may be thrown out, with the simple statement that Paramecium does not so turn.

There remains then only the mechanism of the negative reactions to be elucidated. We may first proceed to an examination of the method by which Paramecium succeeds in keeping out of a drop of some chemical to which it is negatively chemotactic, as in Fig. 4. For this purpose a drop of $\frac{1}{5}$ per cent sodium chloride solution may be selected. To understand the negative reaction, it is necessary to recall the animal's normal method of progression through the water. The unstimulated Paramecium swims nearly straight forward, though in a slightly sinuous course, the narrower, blunter end directed forward. At the same time it revolves on its long axis. Usually, but not invariably, this revolution seems to be from left to right, but there is much variation in the direction.

Now, when a Paramecium, swimming as above described, strikes the margin of the drop of sodium chloride solution, it reverses its course, darting straight backward, — the broad, pointed (posterior) end now being in the lead. At the same time, the direction of rotation on the long axis is reversed, the Paramecium revolving (usually) from right to left. Next the animal swings on its short axis, so as to bring the longitudinal axis of the body out of the line of direction in which it was first swimming. Then the infusorian begins to swim forward again, — following a course which lies at an angle to the course it was taking when it struck the drop of sodium chloride solution. Briefly stated, the animal has adopted the very rational course of backing off, turning in a different direction, then proceeding on past the obstacle, — much as any higher animal would have done.

We may here take up two questions, upon one of which we have already a certain amount of evidence, while the second is pressed upon us by the course of events in the reaction last described. The first is, — Do different kinds of stimuli have different methods of affecting the cilia? For example, does perhaps an acid cause the cilia to strike more strongly backward, driving the animal forward; an alkali, on the contrary, cause the cilia to strike more strongly forward, driving the animal backward? In this way, at first thought, the positive chemotaxis toward acids, the negative taxis toward alkalies might be explained. The results detailed thus far have shown, however, that such a view is not tenable for an explanation of positive chemotaxis, since the latter acts only indirectly through negative chemotaxis. Nevertheless, the general question still remains, — applicable, if not to acids and alkalies, perhaps to other chemical or physical agents. To determine the question with certainty, it will be necessary to immerse the Paramecia directly into solutions of various sorts, and observe the activities of the animals under these circumstances. At the same time evidence may perhaps be obtained on the second question referred to above, which is as follows: What causes the *turning away* of the Paramecium in the reaction toward sodium chloride, above described, after the backward course; and what determines the direction in which it turns? An obvious answer of course suggests itself, —the Paramecium turns away because it wants to get around the obstacle, and the direction in which it turns is determined by the position in which the object lies; it turns so as best to avoid the obstacle. This anthropomorphic view may perhaps be tested, and some evidence on our general question gained, by trying the effect of unlocalized stimuli, — stimuli that act upon the entire surface of the animal at once. This may of course be done by the method above mentioned, — complete immersion of the Paramecia in solutions of different kinds. There will then be no " obstacle " to get by, and, from the anthropomorphic standpoint, no excuse for turning in one direction or another.

With these considerations in mind, Paramecia are immersed directly in solutions of various sorts. This is done as follows: a solution is mixed in a watch-glass, then a quantity of water containing Paramecia is taken up with the pipette and injected energetically into the solution. They are quickly mixed thoroughly by the use of the pipette, then examined with the low power of the microscope.

The Paramecia may be first immersed in an alkaline solution, using for this purpose $\frac{1}{200}$ per cent sodium hydrate. Most of the Paramecia will be seen to swim energetically backward. This may be continued a few seconds or more, then many of the animals will be seen to turn and swim forward in a new direction. If a single specimen is watched, it may perhaps be seen to repeat the process of swimming backward, turning, and swimming forward several times; others may swim backward continuously.

Thus it appears that an alkali causes a reversal of the motion, followed by turning and swimming forward.

Let the Paramecia now be immersed in $\frac{1}{50}$ per cent acetic acid. As in the sodium hydrate, the Paramecia dart backward, only here the motion is more swift. The action of the acid seems much more energetic than that of the alkali; the Paramecia dart furiously backward for an instant, then turn quickly and rush forward for a moment, then perhaps backward again, then turn and rush forward. In a short time the backward motion has ceased; the Paramecia are all swimming forward at a furious rate, and soon they die. The difference in the action of the substances seems due to the fact that acids do not affect Paramecia at all, except when they are so strong as to be severely injurious; hence the great energy of the reaction and the quick death of the animals.

Thus acids also cause the infusoria to swim backward at first. The reaction is qualitatively the same for both acids and alkalies, only the intensity and duration of the different parts of the reaction varying.

In the same way, Paramecia may be immersed successively in a solution of some neutral salt, as $\frac{1}{5}$ per cent sodium chloride; in a solution that is effective only through its strong osmotic power, as a 10 per cent solution of cane sugar; in a watch-glass of water heated to 35° C.; in a watch-glass of water at the freezing temperature. In every case the characteristic features of the reaction are the same. The Paramecia swim backward for a longer or shorter time, then turn more or less, then swim forward. This may be repeated many times. In the different solutions the different features of the reaction may be more or less pronounced, in energy and duration, but the essential nature of the reaction is the same in all.

This series of experiments gives a definite answer to the first of the two questions proposed. Evidently different stimuli do *not* have qualitatively different methods of affecting the cilia. In every case the essential features of the reaction are the same, whatever the agent causing the reaction; only the intensity of the component activities varies. Every reagent causes at first a reversal of the motion of the animal, and therefore beyond doubt a reversal of the motion of the cilia. Since the same result is obtained with stimuli of so heterogeneous and opposite a character, it follows that the determining feature in this method of reaction must be internal. The Paramecium is like a machine in which the wheels are set for a certain motion; to every application of power from whatever source it responds by this motion. It is to be noted, moreover, that while in these experiments the stimulus is continuous, the reaction is discontinuous, the motion being first backward, then sideways, then forward. The demonstration that a single stimulus does not have a single characteristic effect is therefore complete, since each stimulus causes three different motions of heterogeneous and even opposite character.

On the second question, as to what causes the turning away, and what determines the direction of turning, these experiments shed a certain amount of light, though they do not give a determinate answer. Since all these entirely unlocalized stimuli cause the turning away, it follows that the latter is not due to the localization of the stimulus: the Paramecia do not turn from nor toward the source of stimulus, — because they are completely surrounded by and immersed within it. Apparently the turning away is due, like the reversal, to an internal mechanism, not to any characteristic of the external stimulus. Neither can the *direction* in which they turn be determined by an external factor in these cases. It appears probable, therefore, that it is an internal factor that determines the direction of turning.

The next step is to try whether anything can be discerned in the structure of the Paramecium that will give a clue to the factor determining the direction of turning. The most obvious differentiations of the body of the animal are into anterior and posterior ends, and into oral and aboral sides, as shown in Fig. 1. The differentiation of the ends comes into consideration, of course, only in forward or backward motion. Has the differentiation into oral and aboral sides any relation to the turning movement? Owing to the continued rotation of the animal on its long axis, as it swims either backward or forward, it is impossible under ordinary conditions to observe whether there is any relation between this differentiation and the direction of turning. But a clue is given by the conduct of the animals when immersed in a solution of potassium iodide. This reagent has the property of almost indefinitely prolonging each of the component activities in the threefold reaction above described, and affords opportunity for a thorough analysis. When introduced directly into 1 per cent solution of potassium iodide the Paramecia begin, as in other reagents, to swim backward. But in potassium iodide solution this is continued six to eight minutes without change. After this they begin to turn, and this portion of the reaction is continued like the first, so that they spin around on the short axis of the body, as on a pivot, for half an hour at a time. Thus after ten minutes all the Paramecia in the watch-glass will be whirling like tops. It is easy thus to have hundreds of specimens in the field at once with a low power lens, and of these many individuals will be in such a position that it is easy to determine the relation of the direction of turning to the two sides of the animal. It then appears that all are whirling toward the side that contains the contractile vacuoles, — that is, *toward the aboral side.* The direction of motion is indicated in Fig. 6. At first the Paramecia whirl as if on a pivot through the middle of the animal. Later the centre of rotation comes to lie outside the body of the animals, so that they now swim in circles. In every case the centre of the circle lies on the aboral side of the Paramecium, so that the animal is still turning toward the aboral side, though not in such short turns as at first.

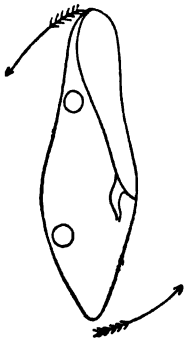

FIGURE 6. Direction of motion of Paramecia whirling in 1 per cent solution of potassium iodide.

Thus under the stimulus caused by potassium iodide, the direction of turning is determined by an internal factor, — the differentiation into oral and aboral sides being the deciding feature. Taking this in connection with the fact that all unlocalized stimuli cause turning, it is a natural inference that the localization of a stimulus has no effect on the direction of turning. As this seems paradoxical and improbable on general principles, however, it must be tested by observation of the reactions of Paramecium toward stimuli that are localized. Is there a difference in the reactions of the animal according as the stimulus affects the anterior end, the oral side, the aboral side, or the posterior end? To determine the answer to this question, it is only necessary to introduce a drop of some chemical compound beneath the cover-glass of a slide preparation swarming with Paramecia, as shown in Fig. 2, and then to watch the edge of the drop with the low power of the microscope. Among the multitudes of Paramecia that strike the margin, many come against it obliquely, or touch it only on one side of the body, and their conduct is then easily observed. The reaction is the same in character as when the animal is immersed in a solution that acts as a stimulus; the Paramecium swims backward a distance, turns, and swims forward. It becomes evident at once that it *does not* turn directly away from the source of stimulus; the position of the drop seems to have little or no relation to the direction in which the Paramecium turns. Sometimes the animal turns in such a way as to carry it away from the drop; in other cases it does not. There is no constancy in the direction of turning, and it is impossible to predict which way a Paramecium meeting the drop at a given angle will turn. In many cases the animal which at first brushed only the edge of the drop is carried, after turning, squarely against it, as illustrated in Fig. 7, in which the numerals indicate successive positions of the animal, while the arrows show the direction of motion. In such a case the whole operation is repeated; the animal backs off, turns in a new direction, and tries again. If the obstruction is large, the Paramecium may be thus compelled to try a dozen times before getting by the obstacle; such cases are often observed.

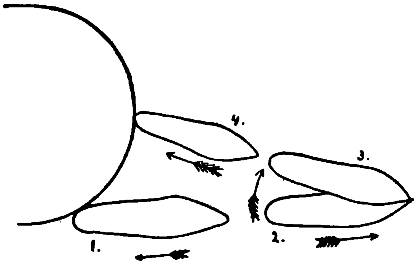

FIGURE 7. Paramecium striking a drop on one edge (1); swimming backward (2); turning (3); and swimming forward squarely against it (4).

This description applies equally well to the case of a mechanical obstruction, and to the reactions toward a very hot or very cold solution.

The inference previously made, that the localization of the stimulus has no relation to the direction in which the Paramecium turns, is therefore confirmed. It remains now to be decided whether the factor which determines the direction of turning when the stimulus is a solution of potassium iodide is the factor in other cases also. Does Paramecium always turn toward its aboral side, whatever be the nature and position of the stimulus?

To answer this question, it is necessary to use the method of observation in gelatine, devised by Jensen. Three grams of gelatine are dissolved by heat in 100 c.c. of ordinary water, and the Paramecia are observed in the thick solution. In this gelatine solution they are able to swim only very slowly, so that all the movements can be closely studied. Any chemical can be mixed with the gelatine solution, in order to observe the effects of unlocalized stimulation, or a drop of any solution can be introduced beneath the cover-glass with the capillary pipette, for observation of the effects of localized stimuli; in fact, the Paramecia may be studied exactly as in water, the only essential difference being that they move more slowly.

The Paramecia are therefore brought into such a gelatine solution, and their conduct toward localized stimuli is observed. The unstimulated Paramecium swims straight forward, revolving slowly on the long axis. On coming in contact with a mechanical stimulus or a drop of some solution toward which it is negatively chemotactic, the animal swims backward very slowly, at the same time revolving slowly from right to left. Then it turns slowly, — *always toward the aboral side.* If the aboral side is above when the turning begins, the animal turns upward; if the aboral side is below, it turns downward; if to the right the animal turns to the right. This is true for a simple mechanical stimulus, for a chemical stimulus of any sort, or for a thermal stimulus; in fact, for any kind of a stimulus that can be tested in the gelatine. After some time in the gelatine solution the Paramecia are usually seen to be somewhat curved, and the curving is always toward the aboral side, as in Fig. 8. A similar curving may often be observed after a strong stimulus, when the Paramecia are mounted in water. The anterior end thus curved of course acts like the rudder of a boat in turning the course of the Paramecium toward the aboral side. Nevertheless, while it aids, it is evident that this is not the only factor in the turning, since Paramecia under other conditions are seen to turn sharply, or even to revolve almost as if on a pivot, when the body is not observably curved at all.

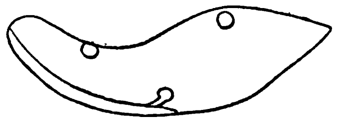

FIGURE 8. Paramecium curved toward the aboral side, under the influence of a powerful stimulus.

The invariable turning toward the aboral side may, as I have since discovered, be demonstrated, without the use of gelatine, in the following way: Paramecia intermingled with masses of bacterial zoöglœa are mounted on a slide and covered with a cover-glass, which is closely pressed down, so as to leave only a narrow space between slide and cover. It will usually be found that many Paramecia are imprisoned in small crevices in the bacterial zoöglœa, so that they can swim only from one end of the crevice to the other, and at each end they must turn. The cover-glass is so close to the slide that the animals can turn only in a horizontal plane. By watching the Paramecia as they turn at the end of the crevice, it will easily be seen that they invariably turn toward the aboral side.

We find then that the question last proposed is to be answered affirmatively. Paramecium after reversing *always turns toward the aboral side,* whatever be the nature and position of the source of stimulus.

A certain feature in the normal straightforward swimming of the unstimulated Paramecium appears to be connected with this fact. As has been often noted, the course in which an individual swims is not perfectly straight, but, as seen from above, the animal seems to swerve from side to side. This swerving has been figured by Verworn[1] and others. The actual path is a narrow spiral, the animal, of course, swerving up and down as well as from side to side. Now, observation of slowly moving individuals shows that at any point in this spiral course the aboral side is on the outside of the spiral. In other words, the Paramecium as it swims continually swerves toward the aboral side. As at the same time it rotates on its long axis, the position of the aboral side continually changes, so that the resulting path is a spiral one, and the general result is the same as if the Paramecium had progressed in a straight line. These facts are indicated in the accompanying diagram of the path of a Paramecium (Fig. 9). Apparently the method of reacting to a stimulus by turning toward the aboral side is a mere accentuation of the ordinary swerving toward that side.

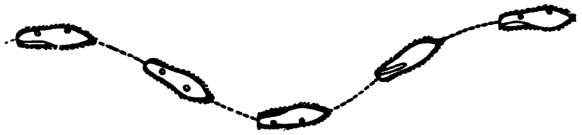

FIGURE 9. Path of a Paramecium. The aboral side is everywhere directed toward the outside of the spiral.

It will here be necessary, in order to avoid misunderstanding, to give an account of an apparent exception to the rule that the Paramecium after stimulation always turns toward the aboral side. In my previous paper on the reactions of Paramecium (*loc. cit.*), I gave an account of the thigmotactic reaction of Paramecium; that is, the reaction due to continued contact with a solid body. I must here supplement the account by setting forth a little more fully certain features in that reaction.

On page 303 of my previous paper is figured a Paramecium resting with its anterior end against a solid body, and exhibiting the currents caused by the cilia under these circumstances, — that is, the currents characteristic of the thigmotactic reaction. As described in detail in the paper cited, the cilia in the oral groove at such a time beat strongly backward, driving a swift current of water toward the rear and causing a vortex to form on the oral side of the anterior half of the animal, as shown in Fig. 19 (*loc. cit.*), while the cilia on the remainder of the body are quiet, or possibly have varying motions tending to counteract the motion of translation that might be induced by the cilia in the oral groove. Meanwhile, the body of the animal remains at rest. This type of activity of the cilia is especially characteristic for resting Paramecia. But sometimes the solid body which causes the thigmotactic reaction is very small and movable, and so does not restrain in any way such motions of the animal as might naturally result from the activity of the oral cilia. In this case the Paramecium moves through the water in a peculiar way, carrying with it the small particle that has induced the thigmotactic reaction.

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[1] VERWORN, M.: Psychophysiologische Protisten-Studien, 1889, p. 116.

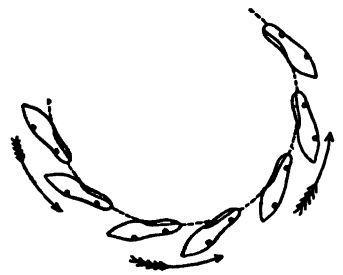

FIGURE 10. Motion of Paramecium in the thigmotactic reaction to a movable particle.

"The cilia beating strongly backward in the oral groove of course impel the animal forward. But as the active cilia are all on one side, there is also a tendency to move toward the opposite side. The resultant of these two motions at right angles to each other is a motion in the circumference of a circle. In fact, the animal moves forward in exactly the lines indicated by the arrows in Fig. 19, only, of course, in the opposite direction from the water currents. The ciliary motion is thus the same whether the thigmotactic animal is at rest or in motion; in the former case the water currents move obliquely backward; in the latter case the animal moves on the same lines obliquely forward. The Paramecium is, as it were, whirled about in its own whirlpool" (*loc. cit.;* p. 305). An inspection of the Fig. 19 referred to in this extract shows that when the animal moves in a circle as there described, the oral side of the Paramecium must look continually toward the centre of the circle. Fig. 10 shows the successive positions of a Paramecium exhibiting the thigmotactic reaction toward such a movable particle; the arrows indicate the direction of motion of the Paramecium, while the circle indicates the path traced by the anterior end of the animal. By reference to this figure it will be noticed that under these circumstances the body moves forward tracing a curve, with the oral side always toward the inside of the curve; in other words, the animal continually turns *toward the oral side.* In connection with the other movements described in these pages, it is important to recall the fact that Paramecia will be sometimes seen thus turning toward the oral side; this, however, is always in the thigmotactic reaction. It is especially frequent when Paramecia are observed undisturbed in water containing many bacteria; when the Paramecia are excited by some unusual condition this reaction is not seen. Now, it is chiefly the reaction under some exciting stimulus that forms the subject of this paper. The thigmotactic reaction is characteristic for resting individuals, and the locomotion in thigmotaxis under certain circumstances in the manner shown in Fig. 10 is an accidental matter, due to the mobility of the solid inducing the reaction. Hence, this is not properly to be accounted a motor reaction, and it is, therefore, with this brief description, left out of consideration in my discussion.

Returning, therefore, to the proper motor reactions, the final step must now be taken; the mechanism of the reactions already described must be determined in terms of the movements of the cilia. All movements of the Paramecia result from motions of the cilia, and the mechanism of the reactions cannot be considered as analyzed into its simplest components until we know what motions of the cilia are at the basis of the general body movements. For studying the motions of the cilia two methods may be used. The first is the observation of the animals in the gelatine solution in the way already given. The thickness of the solution makes the motion of the cilia much less rapid, so that the way in which they move can be determined more or less satisfactorily. The data gained in this way may be supplemented and controlled by the second method of study. This consists in observing the Paramecia in fluid containing powdered carmine. The movements of the particles of carmine in the neighborhood of the animals show the currents caused by the cilia, and from the direction of these currents the direction of stroke of the cilia causing them may be inferred.

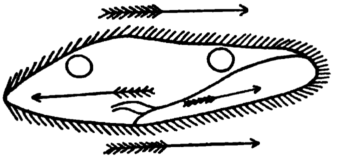

FIGURE 11. Paramecium with cilia reversed. The large arrow in the body shows the direction of locomotion of the animal; the other arrows indicate the direction of the currents of water.

In the unstimulated Paramecium swimming forward the effective stroke of all the cilia is backward, urging the animal forward; the cilia as seen in gelatine give the optical effect of being directed backward, as shown in Fig. 1. On coming in contact with an effective source of stimulus, the cilia are reversed in position, now striking more strongly forward, and presenting the appearance of being directed toward the anterior end, as in Fig. 11. As a result the animal is, of course, impelled backward, or toward the broad, pointed end. It is worthy of especial notice that the cilia in the oral groove are, like all the others, now striking forward, so that a current of water passes swiftly *from* the mouth toward the anterior end, — contrary to the usual direction.

Next, after the backward swimming caused by the reversal of the ciliary motion has ceased, the animal begins to turn. At this stage the cilia are found to have the following motions. The reversal of the cilia in the oral groove has ceased, and they are striking backward again, — thus tending to drive the animal forward. The remainder of the cilia on the anterior half of the animal strike *transversely toward the oral side*. This is shown in Fig. 12. This reaction was first seen clearly in Paramecia that were immersed in a $\frac{2}{5}$ per cent solution of chrome alum; it was then confirmed for other substances and for stimuli other than chemical, by the use of the gelatine solution and by observation of the currents about Paramecia in water containing powdered carmine. The arrows in Fig. 12 indicate the direction of the water currents produced by the cilia at this stage of the reaction. In the oral groove there is a strong current backward, while on the anterior half of the aboral side there is a transverse current from the aboral to the oral side. This motion of the cilia of course causes the Paramecium to turn toward the aboral side (opposite the arrows in Fig. 12), and with this observation we have determined the real mechanism of the turning.

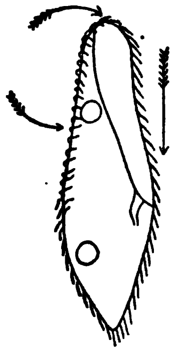

FIGURE 12. Position of the cilia as the Paramecium turns. The arrows indicate the direction of the water currents.

The next step in the reaction is the cessation of the transverse stroke of the aboral cilia, so that they strike backward, as before the stimulation, driving the animal straight forward as at first.

Thus the reaction of Paramecium to any ordinary stimulus is as follows: (1) The cilia over the entire surface of the body are reversed, striking forward, thus driving the animal backward. At the same time its direction of revolution on the long axis is reversed. (2) The reversal of the cilia ceases; those in the oral groove strike backward as at first, while those on the remainder of the anterior half of the body strike transversely, toward the oral side. This results in turning the anterior end toward the aboral side. (3) The cilia all strike backward as at first; the animal, therefore, swims straight forward again. Coincidently with (1) and (2) the body of the animal may be bent toward the aboral side, still further aiding in turning in that direction. At the end of the entire manœuvre the Paramecium is swimming forward along a course which lies at an angle to the course in which it was first swimming. The complete reaction of Paramecium to an ordinary stimulus is represented in Fig. 13. The rotation on the long axis could not be represented in this diagram, but all the other essential features of the reaction are shown.

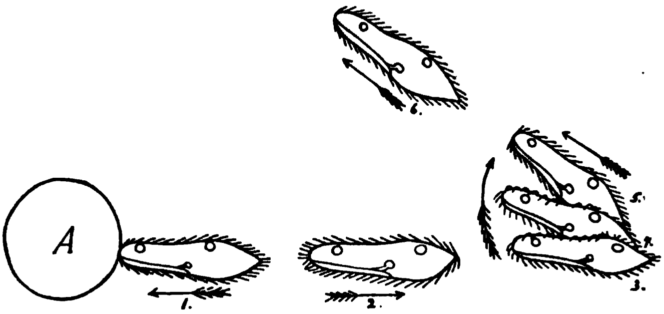

FIGURE 13. Complete reaction of a Paramecium. *A* is a source of stimulus. The numerals give the successive positions of the animal, while the arrows show the direction of motion.

This reaction is not characteristic for any particular class of stimuli, but it occurs in a more or less marked manner whenever any stimulus acts upon the animal with sufficient power to cause a motor reaction. Toward acids and alkalies, toward neutral salts and toward fluids that are active only through their high osmotic pressure; toward heat and cold and mechanical shock, the same reaction is given. Apparently a strong electric current has a related effect, since Verworn has observed that when a very strong electric current is passed through water containing Paramecia, they all swim backward, and thus collect at the anode instead of at the cathode, as is usual with a weaker current. A re-examination of the phenomena of electrotaxis in the light of the general reaction-plan described in this paper appears desirable to determine the relation of the two. Geotaxis, or the reaction of Paramecium relative to the direction of the pull of gravity, in virtue of which they usually rise to the top of a vessel of water containing them, seems more difficult to bring into relation with the general reaction above described. In view of the divergent theories of Jensen,[1] Davenport,[2] and others, the cause of geotaxis cannot be considered certainly known; a reinvestigation of the phenomenon in Paramecium is needed, in the light of the general method of reaction described in this paper, as well as in view of the previously unsuspected positive chemotaxis toward carbon dioxide (as described in No. I of these Studies) — a substance the distribution of which in the water profoundly influences the movements of the Paramecia. Like Miss Platt,[3] I find that the Paramecia of this region do not show clear geotaxis.

Almost the only variation in this entire reaction is in the intensity of its several component activities. To a very weak stimulus, such as a mechanical obstruction in front, the Paramecium responds merely by a reversal of the cilia lasting but an instant and carrying the Paramecium backward but a fraction of its own length. The accompanying reversal of the direction of rotation on the long axis may suffice to turn the aboral side but a few degrees from its original position. The reversal is followed by a momentary transverse stroke of the aboral cilia, turning the Paramecium through but a small angle toward the aboral side. It then swims forward as before. The whole operation has caused a hardly perceptible stoppage in the course of the Paramecium. On the other hand, coming in contact with some strong chemical, such as a drop of $\frac{1}{100}$ per cent sulphuric acid, induces an intense and long sustained reaction. The Paramecium darts swiftly backward many times its own length, revolving rapidly on its long axis from right to left. Finally, the transverse stroke of the aboral cilia intervenes, in an equally powerful manner, and the animal turns sharply. It may thus turn completely around; or in some cases it may whirl more than 180°, so that the immediately following forward course takes it back again into the drop of acid. Certain chemicals exaggerate all the activities composing the reaction to a remarkable degree. Thus in $\frac{3}{4}$ per cent potassium iodide, as already described, the reversal of the cilia lasts six to eight minutes, the Paramecia all this time swimming swiftly backward; then the transverse stroke of the aboral cilia lasts perhaps half an hour, the animal all this time whirling toward the aboral side. In such cases the reaction loses all semblance of an attempt to escape or pass by an obstruction. In other chemicals each component part of the reaction is little exaggerated, but the entire operation is repeated many times, alternations of swimming backward, turning, and swimming forward again continuing for fifteen minutes or more. Different chemicals have a most varied effect on the reaction as a whole, though in every case the effect may be expressed as a modification of the intensity or duration of one or more of the component activities.

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[1] Jensen, P.: Archiv f. d. ges. Physiol., 1893, liii, p. 428.

[2] Davenport, C. B.: Experimental morphology, Part I.

[3] Platt, Julia B.: American naturalist, 1899, xxxiii, p. 31.

The absolute direction in which the Paramecium turns after its backward course is evidently determined by the position of the aboral side when the cilia on that side begin to strike transversely. The position of the aboral side at that time is a matter of chance; the animal revolves continually on its long axis, so that the position of the aboral side is continually changing, and the actual direction in which the Paramecium turns depends upon where the aboral side happens to lie when the reaction reaches the stage in which the aboral cilia begin to strike transversely. Of course this may carry the animal directly into the source of stimulus again; such cases are often observed, as has already been described and illustrated in Fig. 7. In many cases the Paramecium repeats the reaction several or many times before it succeeds in getting by the obstacle. The whole proceeding is an apotheosis of chance. If the source of stimulus is small, the process of drawing back and turning in any chance direction is likely to avoid it. Or, if it is not avoided the first time, the continual revolution on the long axis is likely to bring the aboral side into a new position next time, so that the Paramecium turns in a new direction. If this is continued long enough, a small obstruction is certain, by the laws of chance, to be evaded and passed after a sufficient number of trials. If, on the other hand, the source of stimulus is large; if, for example, the space containing the Paramecium is bounded along one whole side by a solution toward which the Paramecium is negatively chemotactic, then the reaction is repeated until by the laws of chance the course of the Paramecium is so directed as to lie in that part of the space which does not contain the repellent solution. In the case of a solution toward which the Paramecium is strongly negatively chemotactic, chance may be aided by the fact that the intensity of the stimulus usually causes an intense reaction, so that the Paramecia swim a considerable distance backward (which in itself, of course, increases the chance of avoiding an obstacle after turning), and also turn strongly, — perhaps nearly 180°, so that the remainder of the path lies in quite a new direction. It seems probable that for most circumstances in the normal daily life of a Paramecium, this rough hit-or-miss method of reaction is quite sufficient to keep the animal out of danger and to enable it to avoid obstacles. But the utility of the reaction is left to chance, and so circumstances may arise in which the chances are against the Paramecium. Thus in the presence of a drop of some strong chemical, such as $\frac{1}{100}$ per cent sulphuric acid or cupric chloride, it will readily be seen that the chances of escape are limited. The animal responds to the first stimulus by a violent reversal of the cilia, carrying it some distance backward. If now the direction in which the transverse stroke of the aboral cilia carries it after this backward course is by chance such as to take the animal away from the drop, well and good; if, on the other hand, the Paramecium is carried a second time against the drop, it is likely to succumb at once to its poisonous effects. Observation shows that when a drop of such a substance is introduced into a preparation of Paramecia on the slide, it soon becomes surrounded by a zone of the dead infusoria. The same is true of a drop of a dense solution of cane sugar, which is injurious from its osmotic pressure. The Paramecia react when they enter such a dense solution, but the reaction is not precise enough to take them out of the destructive area before they are killed.[1]

The uselessness of the reaction seems especially striking when the Paramecia are completely immersed in a solution that acts as a stimulus. Under these circumstances, when there is no obstacle to be avoided and the stimulus is not localized at all, the Paramecia respond with exactly the same reaction. Paramecia do not modify their conduct to suit the needs of the case, but give the same reaction under all circumstances. The reaction is exactly such a one as might be maintained by natural selection, acting under an environment that makes no severe demands. Since the Paramecium usually swims forward, a majority of the stimuli that affect it at all will affect the anterior end. The whole reaction to *any* stimulus is, therefore, based upon this position of the source of stimulus, without regard to the conditions in a given particular case. For stimuli acting upon the side of the body, the reaction is, therefore, a very imperfect one, the comparative rarity of such stimuli having led to no development of a special mechanism to suit that case. A reaction such as would permit a direct turning away from a stimulus acting upon any side of the animal would evidently involve a much more complicated mechanism, and imply a higher condition psychologically than this simple one-reaction plan. But even this serves, if repeated a sufficient number of times, for avoiding sources of stimuli acting upon the sides of the animal, and under some conditions the result is produced in a fairly direct manner. In certain cases, when the stimulus causing the reaction is very weak, and the Paramecium comes against the source of stimulus very obliquely, so as to touch it only on one side, there appears to be an almost direct turning away, toward the side opposite the stimulus. This is to be observed particularly in the collections of Paramecia in a region containing carbon dioxide excreted by themselves. A long narrow area may thus be formed beneath the cover-glass (as shown in Fig. 14), in which the Paramecia swim back and forth; to the water outside this area they are weakly negatively chemotactic, so that when they swim against the boundaries of the area, the above-described reaction takes place, in a not very pronounced manner. A single Paramecium swimming lengthwise of this area may strike against its lateral boundaries with one side or the other, when it will frequently (not always) be seen to apparently sheer directly off, away from the boundary, and keep on its course undisturbed. Careful observation shows that such cases are to be placed in two categories. (1) When the Paramecium comes against the boundary, the aboral side by chance already lies toward the interior of the area, so that all that is necessary is for the Paramecium to react in the usual manner, in order to leave the boundary. (2) The more interesting case is where the Paramecium comes against the boundary with the aboral side *not* toward the interior of the area. In this case it will be seen to hesitate an appreciable interval before turning away from the boundary. During this moment of hesitation it *starts* to turn toward the aboral side, which is thereby pushed further into the outer fluid, toward which the animal is negative. This induces a faint reversal of the cilia (in agreement with the general

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[1] There is great variation in the repellent power of different chemical compounds. This subject will be treated by the author in a separate paper.

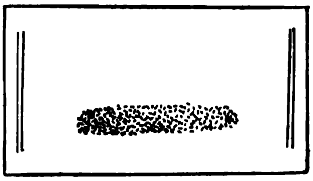

FIGURE 14. Group of Paramecia in a region containing carbon dioxide excreted by themselves.

plan of reaction above described), so that the animal does not progress further in that direction, and may be seen to move backward a barely perceptible distance. It then tries the turning again. On account of the continual rotation on the long axis, the aboral side will now be in a new position; the animal will, therefore, turn in a new direction, and may swim victoriously into the area; if not, a new trial is made during another instant of seeming hesitation. The delay cannot be longer than it requires for the Paramecium to rotate half a turn on its long axis. The animal makes the appearance of feeling about with its anterior end, to discover the proper course to pursue; it is really trying all the time to turn toward the aboral side, but cannot go forward until this carries it into a medium that does not cause a reversal of the cilia. The direction in which the animal shall go is not selected by the Paramecium itself, but is *automatically* selected through the fact that this direction does not cause a reversal of the cilia.

This more direct method is, however, only possible in cases where the negative stimulus is very weak, so as to cause only a very faint reversal of the cilia. With a stronger stimulus the animal shoots back as much as half its length, or more, so that it is now separated from the source of stimulus by a perceptible interval (as in Fig. 13); it is, therefore, free to turn in any direction. Hence, it as frequently turns at first *toward* the source of stimulus as in any other direction. Moreover, even with the weakest stimulus, not all individuals turn in the more direct way just described; many individuals in such an area as shown in Fig. 14 react essentially as shown in Fig. 13. The latter figure shows the *typical* reaction, of which the method just described is a less usual special case.

For the case of complete immersion in a solution that acts as a stimulus, the reaction seems under experimental conditions to be ludicrously imperfect and useless. Under the normal conditions, however, it perhaps serves the Paramecium very well even for this case. Suppose that the Paramecium in its headlong course finds that it has entered completely a circumscribed region of solution having injurious properties. Evidently, to immediately reverse the cilia and swim a long distance straight backward is as likely to rescue the Paramecium from its perilous position as anything it could do, and if this straight course backward does not bring it at once out of the injurious solution, it is perhaps likewise sound practice to change the direction of motion. As under other circumstances, the reaction depends upon chance for success, but the chances are fairly favorable. It is interesting to see how so simple and invariable a method of reacting can meet so well the demands made upon it.

Finally, for the very rare case of stimuli acting upon the posterior end, no provision whatever seems made. It is only when swimming backward in response to a stimulus from in front that a stimulus is likely to act upon the posterior end, — and this is apparently so rare that it can safely be neglected. In cases where animals swimming backward come in contact with something that would act as a stimulus if it affected another part of the body, the cilia continue to strike forward. The normal reaction would be of course to reverse the stroke of the cilia, making them strike forward; as, however, they are already striking forward, there is now no change. Thus Paramecia swimming backward may often be seen to press the posterior end against a mechanical obstruction met in the backward course, and remain there, the forward stroke of the cilia driving them continually against it. Or in the case of chemical stimuli, Paramecia swimming backward often cross the boundary between two substances, when this boundary seems an insuperable obstruction to individuals swimming forward. This is particularly striking when a drop of weak acid is introduced into a slide of Paramecia. As detailed in my previous paper (*loc. cit.* p. 269, Figs. 7 and 8), the Paramecia gather in a ring about such a drop, and are then negatively chemotactic to the inner, more strongly acid part of the drop, and also to the outer fluid. Therefore, they swim about in a ring of some width, surrounding the drop of acid; if they strike against either the inner or the outer boundary of this ring, the cilia are reversed, so that the animals return into the ring. But if a Paramecium on striking the drop of acid at the inner boundary of the ring reverses its cilia strongly and swims backward, it may, if it reaches the outer boundary while swimming backward, cross this boundary without the slightest hesitation, and swim away into the surrounding water, though if it had come against the outer boundary while swimming forward, it would have been at once forced back into the ring by the reversal of its cilia. In cases, however, where a Paramecium swimming backward comes in contact with a source of very strong stimulus, the next step in the reaction is induced, — the Paramecium begins whirling on its short axis, — a proceeding which serves a purpose as little as would the continued swimming backward.

The collection of Paramecia in the region of a given attractive stimulus, as in positive chemotaxis or positive thermotaxis, evidently depends equally upon chance. Nothing acts as a direct attraction to Paramecia. Their habit of swimming continually through the water is what must be relied upon to bring them into the sphere of activity of any agency. In this seemingly random swimming they are frequently met by influences which cause in a slight degree the reaction I have described, as is shown by the frequent slight jerks backward, followed by a slight turn in a new direction. In this way the Paramecium follows the line of least resistance, as it were, and finally may come into the region of a chemical toward which it is positively chemotactic, or into a region of optimum temperature. If now it proceeds on its course across the optimum territory, on coming to the farther boundary it passes into a region of such a nature as induces the negative reaction; the animal turns away and after some repetitions is directed back into the optimum region. The actual process by which this takes place in the case of thermotaxis may be detailed, since my account differs so completely in principle from that of Mendelssohn,[1] who first gave a careful description of thermotaxis, — and since this process affords a very striking example of what might be called the automatic selection of movements.

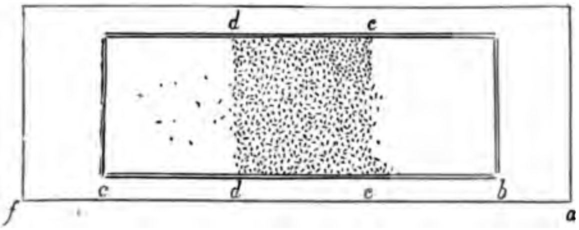

FIGURE 15. Thermotaxis of Paramecium. The right end from *a* to *c* rests upon a water bath heated to 40° C.; the opposite end, from *f* to *d*, rests upon ice.

Four strips of capillary glass tubing, enclosing a rectangular space, are fixed on a slide by means of Canada balsam (Fig. 15); the enclosure is then filled with water containing Paramecia, and covered with a long cover-glass. The Paramecia are scattered uniformly throughout the enclosure, swimming in all directions (the two ends being at first not empty, as they appear in the figure). Now one end of the slide for about a fourth of its length (from *a* to *c*) is allowed to rest on a water bath heated to about 40° C., while the other end, from *f* to *d*, rests on a piece of ice. Soon it will be noticed that the Paramecia at the heated end, between *b* and *c*, are in violent motion. They swim rapidly, some forward, some backward, now and again turning quickly and shooting in a new direction. No particular direction is preferred; the animals simply swim swiftly in any chance direction. Now those which by chance cross the line at *c—c*, coming into a region of low temperature to the left of *c*, cease their violent movements, while those which have started in a different direction keep up their headlong course until they also cross the line *c—c*, which they must inevitably do, purely by the laws of chance, if they continue to be active. Thus in time all the Paramecia which were in the heated region at the right of *c* will have crossed the line *c—c*, leaving the heated space empty. Meanwhile, it is not filled again, because any Paramecia to the left of *c* that may swim toward the heated end, react by reversal of the cilia and turning, when they come to the line *c—c* separating the hot from the cooler region. Thus the Paramecia have entirely vacated the region to the right of *c*, simply by keeping up their undirected movements, until by chance a practicable one carries them across the line. At a certain time after the beginning of the reaction a stream of Paramecia will be seen crossing the line *c—c* from right to left, since any other movement in this region causes a reversal of the cilia and turning.

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[1] MENDELSSOHN, M.: Archiv f. d. ges. Physiol., 1895, lx, p. 1.

At the other end, in the region resting on the ice, the cold does not act like the heat in greatly heightening the activities of the animals; it does, however, stop the thigmotactic reaction, so that all the Paramecia are set in motion, though perhaps very slowly. (If the cold is allowed to act suddenly and strongly, the Paramecia are simply benumbed, and remain where they are.) Some of the Paramecia thus swim by chance across the line *d—d;* the reaction is not, however, so quick and sharp as in the case of too high a temperature, and a number of stragglers will remain benumbed in the cold region. But those which are to the right of *d*, whether originally so, or after having crossed the line *d—d* from the cold region, do not cross this line again, since when they come to the line the cold causes a reversal of the cilia and a turning away. The cold region, therefore, gradually becomes emptied of Paramecia, like the hot space at the opposite end of the slide. The Paramecia in the middle region of the slide may now swim undisturbed anywhere between *c* and *d*, but as soon as they attempt to swim across the line *c—c* or *d—d*, the reaction sets in, and they are turned back. After a time, therefore, practically all the Paramecia are gathered in the optimum region between *d* and *c*, while the ends of the enclosure are empty. The result is not due to direct swimming toward the optimum temperature, nor a direct swimming away from the hot or cold region, but by a sort of "automatic selection" of movements. The Paramecia in the two ends keep swimming hither and thither, until a chance movement in the right direction carries them into the optimum zone. Motion takes place in any direction till one is automatically selected by the fact that it brings the Paramecium into new conditions. The analogy of this to the natural selection of structures is obvious.

Mendelssohn, who described the phenomena of thermotaxis in Paramecium without a knowledge of the finer mechanism of the reaction, assumed that the Paramecia turned and swam *directly* toward the optimum region from either end. In a Paramecium approaching a source of heat, the anterior end would thus be slightly warmer than the posterior end. Mendelssohn assumed that the directive control exercised by temperature must be due to this slight difference in temperature between the anterior and posterior ends of the same animal. He found that in a trough 10 cm. long, a difference of three degrees Celsius between the two ends of the trough was sufficient to cause the Paramecia to collect in the warmer end. The average length of a Paramecium being about 0.2 to 0.25 mm., it was easy to calculate that there was a difference in temperature of about 0.01° C. between the two ends of a Paramecium swimming lengthwise in the trough. It was, therefore, concluded that the Paramecia were sensitive to a difference in temperature of 0.01° C. But, as shown above, the method of reaction is much coarser than that assumed by Mendelssohn; the animal swims from one side of the optimum region to the other, not reacting at all until it comes to a region decidedly hotter or colder. The sensitiveness to temperature differences may, therefore, be much less than that given by Mendelssohn. In a trough with the optimum temperature at one end and changing gradually to the other, the only difference that one can be certain of their appreciating is that between the positive and negative limits of their excursions. If a single Paramecium swims back and forth over a stretch of 5 mm. lying between the cooler and warmer regions of the trough, then it must be concluded that it is sensitive to the difference in temperature between points 5 mm. apart; sensitiveness to any less difference is not proved. Similar considerations apply to other sources of stimuli, since the mechanism of reaction is the same for all classes of stimuli. It is evident that the sensitiveness of Paramecium to different agents may have been greatly overestimated.

Moreover, similar deductions as to the sensitiveness of other organisms must be considered insecure until the exact mechanism of their reactions has been determined.

While the general reaction I have described is evidently from certain standpoints an extremely simple one, yet it bears strongly marked the distinctive features of reactions to stimuli as we know them in the higher organisms, — namely, the lack of any direct and apparent relation between the effect and the cause. In studying the activities of inorganic matter, it is possible to establish relationships of cause and effect — often through a somewhat extended chain from the first cause to the final effect — that may be clearly perceived as causal relations, — so that the effect could be predicted from a knowledge of the first cause and the attendant conditions. On the other hand, in dealing with the activities of living bodies, we are confronted with phenomena in which the steps connecting cause and effect cannot be traced; such phenomena are called reactions to stimuli. Evidently, one of the chief objects in the investigation of the activities of living bodies must be the attempt to resolve reactions to stimuli into the chain of causes of which we must believe them to be composed, so that a series of steps can be traced, each satisfactory from a causal standpoint, from the original cause to the final effect. Can we hope by a study of the simplest unicellular organisms to approach such a resolution of life activities into connected chains of cause and effect? As a hypothetical example of such a resolution may be cited Verworn's theory of the chemotactic movements of Amœba toward oxygen; if this theory is true, the relations of cause and effect are clearly traced from the original cause to the final effect. In the case of the comparatively complicated organism Paramecium, it was perhaps scarcely to be expected that any clear evidence of the actual causal relation between stimulus and reaction should be made apparent, and as a matter of fact, we find, from a causal standpoint, an absolute gulf between them. It is not even possible to trace such general relations between the contractility of the cilia and the nature and position of the sources of stimuli as have been assumed by various authors; Jensen,[1] for example, assumes that a stimulus acting upon one side of the Paramecium causes the cilia of that side to beat more strongly, thus turning the animal away from the source of stimulus; some such relation might perhaps have been reasonably anticipated. But we find that the most varied and even opposite stimuli produce the same reaction, and that stimuli at one end, at the side, or acting over the entire surface of the animal at once, produce the same result. The reaction appears to be purely arbitrary, without any relation to the nature of the cause. It evidently depends upon the internal mechanism of the body of Paramecium, and this mechanism is so complicated that we are quite unable to trace the steps which lead from cause to effect.

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[1] JENSEN, P.: Archiv f. d. ges. Physiol., 1893, liii, p. 471.

For all practical purposes, therefore, the reactions of Paramecia present phenomena not a whit simpler for a causal understanding than the activities of a highly developed Metazoan. The mechanism of the unicellular organism, lacking as it does a complex nervous system, may, as a matter of fact, be simpler than that of a Metazoan, but in both cases the mechanism is of sufficient complication to interpose for our understanding an absolute break between stimulus and reaction; hence the study of the Protozoan gives no deeper insight into the essential nature of such reactions than does the study of a Metazoan. Whether the reactions of such a comparatively undifferentiated lump of protoplasm as Amœba will really prove of any more value for such an insight remains to be seen; certainly Amœba is deserving of a thorough experimental study from this standpoint.

Thus the principal intrinsic interest of this investigation lies not so much in the fact that the organism studied is a mass of protoplasm constituting but a single cell, as in the fact that it represents a very low place in the psychological scale. From the psychological standpoint, the reactions of Paramecium are extraordinarily simple. We have in this animal perhaps as near an approach to the theoretical reaction postulated by Spencer and Bain for a primitive organism, — namely, random movements in response to any stimulus, — as is likely to be found in any living organism. The motions are strictly random, so far as the position of the source of stimulus is concerned; the animal always swimming, after stimulation, in the direction of one of its own ends (the posterior), and turning toward one of its own sides (the aboral), without regard to the relation of these directions to the source of stimulus. And by the repetition of the reaction the direction of movement is frequently changed, — always without relation to the localization of the stimulus. It appears not to have been foreseen, theoretically, that such random movements would of themselves if continued carry the animal out of the sphere of influence of the agent causing them and keep it from re-entering. To accomplish this result, it is only necessary that the direction of motion should be changed at the moment when the stimulus begins to act, and at intervals so long as its action continues.

It is evident from this method of reaction that Paramecia are neither directly attracted nor directly repelled by any agencies. It is often assumed that it is a universal property of living organisms to react in two opposite ways toward stimulations, — to be attracted by some and repelled by others. In view of the reactions of Paramecium this assumption must fall to the ground, as certainly not universal, and possibly not nearly so general as has been supposed. The mechanism of the reactions of most simple organisms remains yet to be ascertained.

In regard to the position in the psychological scale to be assigned to Paramecium, the following may be said. The organism responds to any stimulus by a definite, well-characterized reaction. The same may be said of the isolated muscle of a frog. The intensity of the reaction varies with the nature and intensity of the stimulus; this also is true for the muscle. Under certain influences the Paramecium remains quiet; likewise the muscle. The *directive* relations of the motions performed are determined both in the Paramecium and in the muscle by the structure of the organism, not by the localization of the stimulus. There seems, then, no necessity for assuming anything more in order to explain the reactions of Paramecium than to explain the reactions of the muscle. We require, therefore, little or nothing more than irritability, or the power of responding to a stimulus by a definite movement, to account for the activities of Paramecium. Since the direction of motion of the Paramecium has no relation to the position of the source of stimulus, there is no need to suppose that the animal has anything related to a *knowledge* of this position. Moreover, it exercises no *choice*, — the direction of motion being always the same with reference to the parts of the animal. I do not see that we are compelled to assume consciousness or intelligence in any form to explain the movements of this creature.

SUMMARY.

1. Paramecium has a single motor reaction, by which it responds to all classes of stimuli. This reaction consists of the following activities. (1) The direction of the stroke of the cilia is reversed, over the entire surface of the animal, so that they strike forward, driving the animal backward. (2) The reversal ceases; the cilia in the oral

groove strike backward, as at first, while the aboral cilia of the anterior half of the body strike *transversely toward the oral side. This results in turning the animal toward the aboral side.* The anterior end may, if the stimulus is strong, be curved toward the aboral side, thus aiding in turning toward that side. (3) The cilia all strike backward as at first, driving the animal forward again. At the end of the reaction the Paramecium is swimming forward on a path which lies at an angle to the path on which it was moving at first.

2. The intensity and duration of the different parts of this reaction vary with the nature and intensity of the stimulus.

3. Paramecia do not turn directly toward a beneficial source of stimulus, nor directly away from an injurious source of stimulus, but the reaction is always as stated under 1. That is, localized stimuli, acting on one side or one end of the animal, have the same effect as stimuli acting upon the entire surface of the body at once. The direction in which the Paramecium turns after a stimulus is determined by internal factors, and is expressible in terms of the animal's structure; it has no relation to the position of the source of stimulus. The *absolute* direction in which the animal turns is a matter of chance; if it does not carry the animal away from the obstruction the first time, the entire reaction is repeated till it does.

4. *Positive* and *negative taxis*, as applied to Paramecia, are merely convenient terms for expressing the fact that the animals form collections in the regions of certain influences and do not form such collections in others; the terms do not express motor reactions. Paramecia are not directly attracted or repelled by any agencies or conditions. The collections formed in the regions of certain agencies are due to the fact that these agencies cause *no motor reaction.*

5. Different classes of chemical or physical stimuli do not have qualitatively different methods of affecting the stroke of the cilia.

6. In view of the mechanism of the reactions, the sensibility of Paramecium to different agents has probably been much overestimated, since it is not the differential effect on the anterior and posterior ends of the animal that induces the reaction.

7. In the reactions of Paramecium the steps between cause and effect cannot be traced, opposite causes producing the same effect, and the same cause if continued producing opposite effects. The nature of the reaction is conditioned by the internal mechanism of the animal's body.

8. The reactions of Paramecium indicate that the organism stands at the very bottom of the psychological scale. Simple irritability, or the property of responding to a stimulus by a fixed set of movements, seems sufficient to account for its activities.

The results presented in the foregoing paper were obtained in connection with an extended study of the chemotaxis of Paramecium, carried out in the Laboratory of the United States Fish Commission for the Biological Survey of the Great Lakes during the summer of 1898, and continued at Dartmouth College during the following autumn. It is a pleasure to acknowledge here my indebtedness to Prof. J. E. Reighard, the Director of the Survey, and to the officials of the United States Fish Commission for assistance and courtesy in every way in carrying on this investigation, as well as for permission to publish the present paper.

DARTMOUTH COLLEGE,
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STUDIES ON REACTIONS TO STIMULI IN UNICELLULAR ORGANISMS.

H. S. JENNINGS.

III. REACTIONS TO LOCALIZED STIMULI IN SPIROSTOMUM AND STENTOR.

IN the second of these studies (*American Journal of Physiology*, May, 1899) the writer has given an account of the mechanism of the reactions of Paramecium that involves an entirely different conception of the nature of these activities from that which has been generally assumed. It was shown that this protozoan has but one motor reaction in response to the most varied stimuli, and that it reacts without any relation whatever to the position of the stimulating agent, so that it cannot be said to be attracted or repelled by any agency or condition — its reaction being strictly comparable in all essentials to that of an isolated muscle. The importance of this, in case it should turn out to be the general method of reaction for unicellular organisms, is obvious, involving, as it does, the rejection of almost all hitherto received theories of the mechanism of reactions; the question, therefore, immediately arose as to whether this method of reaction was to be extended to other Protozoa. The following study of the reactions of Spirostomum and Stentor is presented as a contribution toward answering this question.

The minute size of Paramecium brought with it the great disadvantage that it was not possible under experimental conditions to apply localized stimuli to definite parts of the body, so that recourse was necessary to observation of chance contacts of sources of stimuli with one or another part of the body; an unsatisfactory method, and one requiring much time and patience. This difficulty is obviated in *Spirostomum ambiguum* and *Stentor polymorphus*, which are both so large that there is no difficulty in applying stimuli to any desired region on the surface of the body. The simplest means of doing this is to touch any point on the surface of the body with a proper instrument, thus giving the animal a sharply localized mechanical stimulus. Other methods are given in the following account of observations.

Spirostomum ambiguum (Fig. 1).

Spirostomum ambiguum is one of the largest of unicellular animals, reaching a length of two or three millimeters. The average length of those on which the following observations were made was about one and one-half millimeters. In form Spirostomum is a long, slender cylinder of nearly equal diameter throughout, but slightly smaller at the ends. The mouth lies behind the middle of the body, and from this a band of large cilia (the adoral zone) runs to the anterior end of the body. The large contractile vacuole lies at the posterior end, and from this a canal runs almost the entire length of the body near its aboral side, or curving a little onto the right side. The adoral zone and this canal form important landmarks for determining directive relations in studying the movements of the animals. The posterior end is truncate, while the anterior end is rounded and shows a difference in its contour on the two sides of the animal. The tip is curved slightly toward the side on which the mouth is situated (the oral side), so that on the opposite or aboral side the contour is a long convex curve, while on the oral side there is almost an angle. These facts are best appreciated in the figure; while not prominent, they are visible, and are of the greatest importance for orientation while studying the movements of the animal. The difference in the two contours of the anterior end possibly does not correspond precisely to the distinction between the oral and aboral sides, as determined by the position of the mouth; the exact relation of parts is very difficult to determine on account of the continual twisting movements of the animal. But the difference in contours in any case marks very nearly the oral and aboral sides, so that when the aboral side is spoken of in the following account it signifies that side of the anterior end in which the curve is longest, while the oral side is that which presents the shortest curve (see Fig. 1). The entire surface of the animal is covered with cilia, arranged in somewhat oblique longitudinal rows.

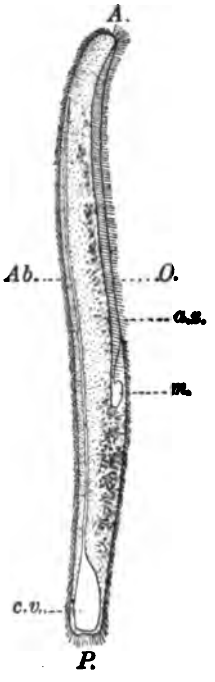

FIG. 1.—*Spirostomum ambiguum*, right side. *A*, anterior end; *P*, posterior end; *O*, oral side; *Ab*, aboral side; *a.s.*, adoral zone; *m*, mouth; *c.v.*, contractile vacuole.

The unstimulated Spirostomum swims forward by means of the backward stroke of its cilia; at the same time it may revolve on its long axis. This revolution is usually from right to left. The body (as shown in Fig. 1) is not quite straight, but a little in front of the middle is slightly bent, so that the anterior part, as the body revolves, describes the surface of a cone. Owing to the animal's continual movement and its power of twisting into a spiral and of bending sharply, taken in connection with the only slight differentiation on the surface of the cylindrical body, the orientation of the body in relation to the direction of movement is very difficult to determine. But by keeping the attention fixed upon the form of the anterior end, as described above, it is possible under favorable conditions to observe that the aboral side of the anterior end is always on the outside of the cone, the oral side looking within. As the animal moves forward in connection with this revolving motion, the path of course becomes a spiral; it agrees exactly with the path of Paramecium, in that the aboral side always looks toward the outer side of the spiral. At times the animal swims some distance without revolving on its long axis; at such times the path is not a spiral.

We will now proceed to a systematic examination of the changes in motion due to stimuli of different sorts and applied at different points on the animal's body.

A. *Mechanical Stimuli.*

The method of study consisted in touching with a capillary glass rod, of less diameter than the animal's body, different points on the surface of the animal, and noting the reactions caused. This procedure presents no difficulties.

1. *Stimulus at Anterior End.* — If the animal is touched at the anterior end with the tip of the glass rod, it immediately contracts strongly, becoming short and thick, and the zones of cilia forming spirals surrounding the body, in the manner well known. (See the figure given by Bütschli in *The Protozoa of Bronn's Klassen und Ordnungen des Thierreichs*, Taf. LXVII, Fig. 2 *b*.) At the moment of contraction it darts backward a little. It then gradually extends, continuing to swim backward. As it swims backward it revolves on its long axis, in all cases observed, from right to left, in the same direction as when swimming forward. Next it begins to turn its anterior half to one side, usually at the same time beginning to swim forward. Like Paramecium, it always turns toward the aboral side of the anterior end. It usually revolves at the same time, so as to describe a very wide spiral, with the aboral side on the outside of the spiral, finally straightening out and swimming forward in the direction indicated by the position of the aboral side at the time it straightens. Briefly, the animal when stimulated at the anterior end contracts, backs off, turns to the aboral side, and swims forward on a path which lies at an angle to the path on which it was previously swimming. In the case of a very slight stimulus the contraction may be omitted, the rest of the reaction being given as usual, ex-

cept that the animal swims only a short distance backward before turning.

2. *Stimulus at Posterior End.*—If now the animal is touched at the posterior end, exactly the same reaction is produced. It contracts, then swims backward, — therefore toward the point of stimulus (whereas in the other case it swam away from the point of stimulus). Then it curves toward the aboral side, describing a wide spiral, and finally swims forward in the direction of the aboral side, exactly as described above. It makes no difference to the reaction which way the animal is swimming when stimulated: if swimming forward, the direction is changed; if swimming backward when touched at the posterior end, it continues to swim backward after contracting.

The posterior end is slightly less sensitive than the anterior, so that a very weak stimulus at the posterior end may cause no reaction at all. No matter where stimulated there are rare cases in which the animal when stimulated merely contracts (always darting back a little at the moment of contraction), then stops for a time, then resumes a forward motion. Out of one hundred cases stimulated at the anterior end, ninety-six reacted in the typical manner, while four gave the incomplete reaction just mentioned. Out of one hundred cases stimulated at the posterior end, ninety-two gave the typical reaction, while eight reacted incompletely. This difference in the number giving the typical reaction is probably a mere statistical variation, which would disappear with larger numbers; if any significance is to be attached to it, it is merely that already noted — that the posterior end is slightly less sensitive than the anterior.

The reaction is thus exactly the same whether the stimulus occurs at the anterior or the posterior end.

3. *Stimulus at One Side.* — When the animal is touched anywhere on the surface of the body between the two ends, the same reaction is given as in the two foregoing cases; the animal contracts, swims backward, curves toward the aboral side, and swims forward toward that side. The final direction in which it swims bears no relation to the position of the side on which the stimulus was given.

4. *Stimulus Unlocalized.* — An unlocalized mechanical stimu-

lus can be given by jarring the dish or slide containing the animals. They contract, swim backward, turn toward the aboral side, and swim forward, exactly as in the other cases.

Thus the reaction given by Spirostomum to a mechanical stimulus is identically the same, whatever part of the body is stimulated, or even if the stimulus is not localized at all. A diagram of the reaction of Spirostomum to a stimulus is given in Fig. 2.

5. *Repeated Stimuli.* — When the same animal is repeatedly stimulated, certain features in the reaction are especially worthy of notice. At the first stimulus the animal contracts, then swims backward. Now if, after recovering from the contraction, but while still swimming backward, it is again stimulated, it again contracts and continues to swim backward. On a third stimulation, while still swimming backward, it usually *reverses* its course and swims forward. A reversal of the direction of motion now usually occurs at each new stimulus up to four or five. This reversal at each new stimulus may easily give the impression that the animal is swimming each time away from the source of stimulation, and hence that it is reacting with relation to the localization of the stimulus; but this appearance is due merely to a psychological peculiarity of the experimenter. It is natural for the experimenter to touch the animal at the end toward which it is moving — to "head it off" as it were. On the third trial he will, as above stated, usually succeed in getting it to reverse its motion and swim in the opposite direc-

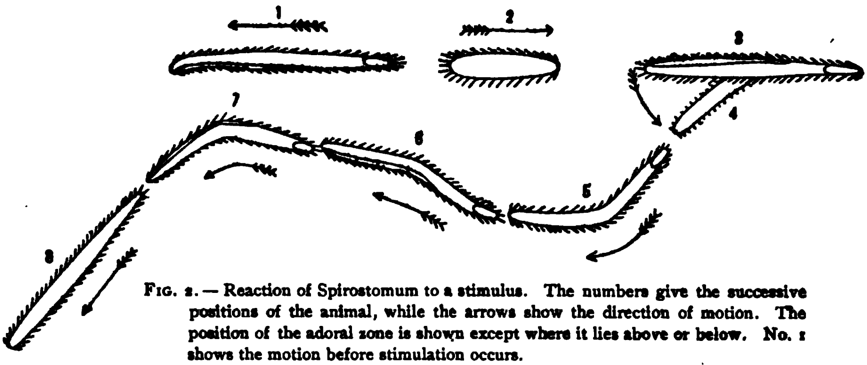

FIG. 2. — Reaction of Spirostomum to a stimulus. The numbers give the successive positions of the animal, while the arrows show the direction of motion. The position of the adoral zone is shown except where it lies above or below. No. 1 shows the motion before stimulation occurs.

tion. He then "heads it off" again by touching the other end toward which it is now swimming, with the result that it reverses again. This alternation on the part of experimenter and infusorian may continue for a number of times, giving the impression that the animal is clearly reacting with reference to the position of the source of stimulus, fleeing from it in each case. But this alternation is really an independent phenomenon in each of the two organisms concerned in the experiment, as is proved by the following. If the experimenter continues to stimulate the animal at the same end, regardless of the direction in which it is moving, the animal's direction of motion will alternate as before. Thus, after the second stimulation, while the Spirostomum is swimming backward, if stimulated at the anterior end, it will now swim forward or toward the source of stimulus; if stimulated again at the anterior end, it will reverse and swim backward; again, and it swims forward once more. In these cases, as in the others, therefore, the direction of motion has no relation to the localization of the stimulus.

If the stimulations are continued after five or six times, the animal will continue to swim violently in one direction or the other, without regard to the repetition of the stimulus or its localization. It is thus possible to stimulate the animal repeatedly at its posterior end while it is swimming violently to the rear, and thus toward the source of stimulus; in other cases it swims as violently away from the stimulus. In no case does the position of the stimulus have any effect on the direction of motion.

B. *Chemical Stimuli.*

It is easy to localize the action of chemical stimuli in the following manner. A capillary glass rod is coated with paraffin. A crystal of NaCl or other salt is then attached to the rod by means of the paraffin coating, and can then be held near to any part of the animal's body.

1. *Stimulus at Anterior End.* — The crystal of NaCl is held close to the anterior end of the Spirostomum, but without touching it, so that only the diffusing salt in solution comes in contact with the animal. The Spirostomum contracts, swims

backward (away from the source of stimulus), turns toward the aboral side, and swims forward, exactly as in the case of a mechanical stimulus.

2. *Stimulus at Posterior End.* — The crystal of salt is held close to the posterior end. The animal contracts and swims backward — therefore *toward* the source of stimulus. It thus either strikes the crystal of NaCl or passes through the densest part of the solution; then continues backward some distance, finally turning toward the aboral side and swimming forward.

3. *Stimulus at the Side.* — The animal reacts as in the two preceding cases, the direction of motion having no relation to the position of the stimulus.

4. *Stimulus Unlocalized.* — The Spirostoma are dropped directly into 2 per cent NaCl. They contract, swim backward, turn about irregularly, and soon die.

The following experiment, giving results in almost all the above categories, is particularly striking. A number of Spirostoma are placed on a slide in a considerable quantity of water. Then a few crystals of NaCl are placed in the center, the cover-glass quickly supplied, and the reactions of the animals noted. All those in the immediate neighborhood of the NaCl soon contract and swim backward, as the flood of salt solution diffusing from the crystals comes against them. The Spirostoma are scattered, with axes oriented in no special direction, therefore some lie with anterior ends directed toward the mass of salt, so that this end first comes in contact with the salt solution; others with posterior end thus directed; others with long axis oblique to the direction of the mass of salt. All contract and swim backward, whatever part of the body is first met by the diffusing solution of NaCl. Those with anterior ends directed toward the mass, swimming backward, of course move directly away from the salt. Those with posterior end toward the salt, likewise swimming backward, pass directly into the densest part of the solution of NaCl, and are quickly plasmolyzed and killed. Those with long axis oblique to the direction of the NaCl also swim backward, some thus approaching more or less obliquely the solution of NaCl; these do not turn, but swim straight on, crossing the area and coming out on the other side, unless plasmolyzed and killed during the passage.

It is evident that the Spirostoma are neither attracted nor repelled by the NaCl; it merely sets in operation their one reaction, and this takes them into danger or safety as chance may direct. Under normal conditions, of course, the anterior end will usually be directed toward the stimulating agent, since the animal generally swims forward, and masses of dangerous chemicals are not often dropped suddenly into the midst of a group of the Infusoria; hence the device of swimming backward usually saves the animal. The following curious experiment shows how possibly a combination of circumstances might arise even under normal conditions such that the reaction would result in the destruction of the animal. A small mass of NaCl was slowly dissolving in the center of the slide. A Spirostomum was swimming forward directly away from the diffusing salt, not being in the region of its influence at all. Its posterior end was thus pointed toward the salt, but as it was swimming away it was in no danger. Now a slight jar was given to the preparation — such as might easily occur in nature. Thereupon the Spirostomum reacted in the usual manner, by contracting and swimming backward. It thus swam toward the NaCl, until finally its posterior end came in contact with the advancing flood of salt solution. Thereupon the customary reaction was again induced still more powerfully; the animal contracted and swam still more swiftly backward; thus entering the salt solution, it was plasmolyzed and killed.

When a Spirostomum swimming forward comes in contact with a diffusing chemical, it contracts, darts backward, then swings its anterior end about, finally turning toward the aboral side and swimming straight forward — so long as this does not take it again into the region of the stimulating agent. If it does, the reaction is repeated until by the laws of chance the Spirostomum is directed into a region which does not cause stimulation. If the stimulus with which the anterior end comes in contact is very weak, the animal may omit the contraction and move a little backward without contracting; then the anterior end is swung about in a circle (the aboral side, of course, toward the outside of the circle), while the animal starts forward. So long as the anterior end is thus carried into a medium which causes the weak stimulus, the forward movement is at once checked and the animal jerks backward again; as soon, however, as the anterior end in its circling comes into a direction such that swimming forward does not carry the animal into a region causing the reaction, the animal continues to swim straight forward. All these facts find a precise parallel in the reactions of Paramecium.

When the Spirostomum is swimming backward, the course is never changed by the circling about of the posterior end and the turning in one way or another, as it is when the anterior end is directed forward; the posterior end seems to have no power of initiating a turning movement. In this connection the reactions of the separate parts of a Spirostomum, cut into two or more pieces, is of interest.

C. *Reactions of Separated Parts.*

There is no difficulty in cutting Spirostomum with scissors or scalpel transversely into short pieces. Any piece with which the anterior end remains in connection, though it be but one-tenth of the entire animal, reacts in essentially the same way as the entire organism — by contracting, swimming backward, turning and swimming forward. Its motion, perhaps, differs a little in degree from that of the entire animal in the fact that turning is more frequent and pronounced, the piece at times swimming in a small circle. The direction of turning is, as in the uninjured specimen, toward the aboral side. Any piece from which the anterior end is separated, while the posterior end is uninjured, reacts as follows. When stimulated, it contracts and swims backward, *does not turn*, but soon swims forward. It swims but a short distance forward, then starts backward again; after going in this direction once or twice its own length, it swims forward about the same distance; then again backward. It continues thus to oscillate back and forth indefinitely. When the anterior end is removed, therefore, the motion takes the form of a rhythmical back and forth movement. This is true when the posterior piece comprises as much as nine-tenths of the entire animal.

It seems, therefore, that the power of initiating a turn, and the power of continuing a course once begun, are localized in some way in the anterior end.

Stentor polymorphus (Fig. 3).

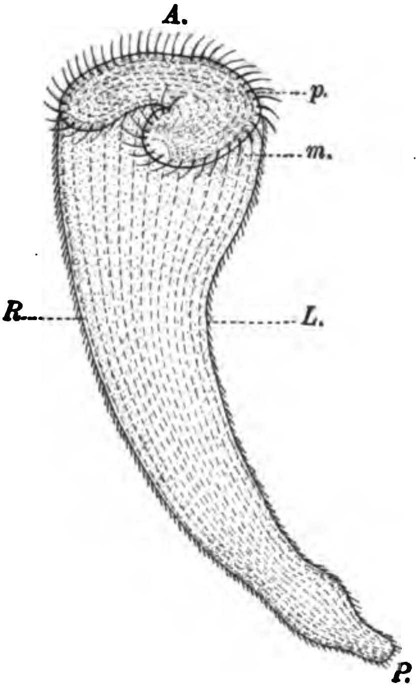

FIG. 3.—*Stentor polymorphus*, partially contracted free-swimming individual, oral surface. *A*, anterior end; *P*, posterior end; *L*, left side; *R*, right side; *m*, mouth; *p*, peristome.

Stentor polymorphus is smaller than *Spirostomum ambiguum*, but is still of sufficient size to make the application of localized stimuli a matter of no difficulty. It is a trumpet-shaped animal, exceedingly changeable in exact form and proportions. Fig. 3 shows a usual form of the animal when swimming freely; when anchored by its base the form is more extended and slender. The surface of the animal is covered with cilia in longitudinal rows, while the broad anterior end, known as the peristome, is surrounded by a circle of larger cilia forming the adoral zone. At one side of the disk is a funnel-like depression which leads into the mouth. That surface of the body nearest to which the mouth lies may be called the oral surface; the opposite one, the aboral surface. Considering the oral as equivalent to ventral surface, we may define right and left sides as follows: When the oral surface is below, and the anterior end is away from the observer, the right and left sides of the animal correspond to the observer's right and left. (In the figure the oral surface is above, so that right and left sides are reversed.) The body of the animal is usually curved, being bent from the direction of the anterior end toward the left side; sometimes there is near the posterior end a second short curve to the right.

Stentor polymorphus is often found attached by its posterior end; at other times it swims freely in the water. The motion in the free-swimming individuals is as follows: The animals swim slowly forward; at the same time they may or may not revolve on the long axis. The revolution when it occurs is usually, if not invariably, to the left. When the animal swims forward without revolving on its long axis, the path is usually a curved one, the animal continually swerving toward its right side. In this way the Stentors usually describe circles of greater or less radius. If they revolve as they swim forward, they continually swerve to the right also; but owing to the revolution, the right side continually changes its position, so that the path becomes a spiral one, as in Paramecium and Spirostomum. The motion of *Stentor polymorphus* is usually very slow, so that all these relations are observable without the slightest difficulty.

A. *Reactions to Mechanical Stimuli.*

The animals were stimulated in the same way as Spirostomum, by touching them at any desired point with a capillary glass rod.

1. *Stimulus at Anterior End, on Peristome.*— The animal contracts and swims backward a short distance (its own length or a little more). As it swims backward it revolves on its long axis to the left — in the same direction as when swimming forward. Then it turns on its short axis to its right and swims forward.

2. *Stimulus at Side.*—Identically the same reaction is given as when stimulated at the anterior end; the animal contracts, swims backward, turns to right, and swims forward. The turning is not with reference to the position of the source of stimulus, but is always toward the right side. Therefore, if

stimulated on the right side, the animal turns toward the side stimulated; if on the left side, it turns away from the side stimulated. Owing to the revolution on the long axis while swimming backward, the position of the right side at the time of turning on the short axis bears no definite relation to its position at the time of stimulation; so we find that the absolute direction toward which the animal turns has no constant or prevailing relation to the absolute direction from which the stimulus came.

3. *Stimulus at the Posterior End.* — The posterior end of Stentor, narrowing to a point of attachment, is very little sensitive, so that touching it with the rod of glass usually causes no reaction whatever. By giving it a smart blow, however, a reaction can be induced, and this is then identical with the reaction already described. The animal thus, of course, swims at first *toward* the source of stimulus.

4. *Stimulus not Localized.* — If the vessel containing the Stentors is jarred, they react in the same manner as to localized stimuli.

B. *Chemical Stimuli.*

Stentor gives the same reaction to chemical as to mechanical stimuli. If when swimming forward through the water it comes in contact with a chemical substance sufficiently powerful to act as a stimulus, it contracts, swims backward, turns to the

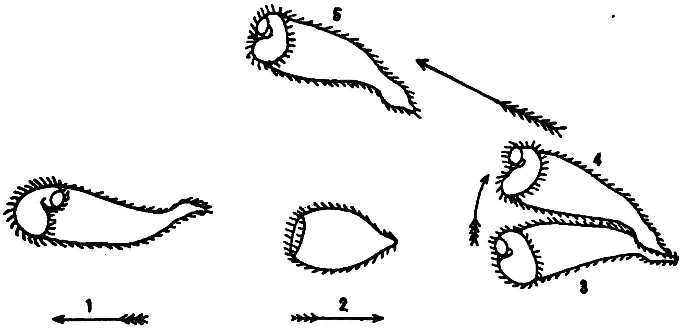

Fig. 4. — Diagram of the reaction of Stentor. The arrows show the direction of motion, while the numbers indicate the successive positions of the animal; No. 1 showing the animal before stimulation occurs.

right, and swims forward on a new path; if this path takes it again into the stimulating region, the reaction is repeated; thus by the laws of chance the animal will in time probably be brought into a region which does not act as a stimulus. The reaction is the same whether the stimulus is localized or is a general one. The reaction is in essentials like that of Paramecium and Stentor.

The reaction of *Stentor polymorphus* is shown in Fig. 4.

SUMMARY AND CONCLUSIONS.

The reactions of Spirostomum and Stentor are similar in all essentials to those of Paramecium. To any stimulus all these animals respond by swimming backward, turning to one side, and then swimming forward. Paramecium and Spirostomum always turn toward the aboral side; Stentor, toward the right side. In Spirostomum and Stentor a contraction of the body forms an additional feature of the reaction. The reaction is not modified in any way by the position of the stimulus; the direction of motion is the same whether the source of stimulus is at the anterior end, the posterior end, the side, or if the stimulus is not localized at all. If the stimulus is at the anterior end, the animal necessarily swims away from it; if at the posterior end, it swims toward it, even when this results in the destruction of the animal. The discussion and conclusions given in my previous paper (*loc. cit.*) in regard to Paramecium are, therefore, equally applicable to Spirostomum and Stentor, and need not be repeated here.

The fact that three such dissimilar ciliates as Paramecium, Spirostomum, and Stentor agree in their reaction in all essential particulars certainly raises a presumption that the mode of reaction is of this general character throughout the ciliate Infusoria. This is especially probable in view of the fact that the revolution on the long axis and progress in a spiral course, which plays so essential a part in these reactions, and indeed seems to have the special purpose of making such a method of reaction possible, is already known to occur in the motions of almost all ciliates. Moreover, the same is known, through the researches of Nägeli, to be true of the flagellate swarm spores of plants. According to this investigator, such swarm spores swim in a spiral course, with the same side always toward the outside of the spiral — exactly as I have described for Paramecium, Spirostomum, and Stentor. Another observation of Nägeli renders it extremely probable that the mechanism of the reactions of these organisms is essentially the same as in the three ciliates named. When these flagellate swarm spores strike in their forward course against an object, they cease the forward motion for a time, but continue to turn on the long axis; then "there ensues a backward motion, with posterior end in advance, while at the same time they rotate in the opposite direction. This backward course usually lasts but a short time and becomes gradually slower; it is soon exchanged for the forward motion, which takes place, as a rule, in a somewhat different direction from the original one."[1] This description would apply without the slightest change to the reactions of Paramecium, Spirostomum, and Stentor under the same circumstances. It may be predicted with much confidence that the reactions would be found to be similar under other circumstances; that the "somewhat different direction" of the swarm spores would be found to be always toward the side which faces the outside of the spiral course, and that the same reaction would be given whatever the nature and position of the stimulus. If this is true, then the conclusions which I have drawn for Paramecium in my previous paper would apply equally to the Flagellata. Certainly until the mechanism of the reactions of these and other unicellular organisms is determined by observation, it is hardly worth while to base any conclusions on theoretical schemes of the character usually given for such reactions. Theories of the reactions of unicellular organisms may be placed in two general classes: on the one hand are those theories which look upon the activities of unicellular organisms as determined in a manner similar to those of human beings, by a play of desires, motives, etc.; while at the other extreme are theories in which the movements are looked upon as of a character essentially similar to those taking place in a chemical reaction—the protoplasmic mass reacting rather as a substance than as an individual. Some such theory as this latter seems to be latent in the minds of many biologists; it finds typical expression in the scheme for the reactions of a unicellular organism to chemical substances given by Le Dantec (*La Matière Vivante*, pp. 50–54). In this scheme, which is illustrated by geometrical constructions and almost takes the form of a mathematical demonstration, Le Dantec assumes that there is a tension between the chemical in solution and the surface of the protoplasmic mass, and that this tension acts in lines of force directed either away from the center of the protoplasmic mass, or toward that center. The movement of the organism is then due to the difference in this tension on the two sides of the protoplasmic mass — that directed toward the center from which the chemical is diffusing, and that directed away from it. As the chemical diffuses from a center, the solution is less intense the farther one passes from the center; hence the solution is less dense on that side of the protoplasmic mass farthest away from that center. Assuming that the tension caused by the chemical acts on the protoplasmic mass in lines of force directed away from the center of the mass, it is mathematically demonstrable that this force will be stronger on the side toward the center of diffusion of the chemical, and that the resultant of all the lines of force will be a force directed exactly toward this center of diffusion. Hence the protoplasmic mass will move toward the center of diffusion of the chemical; in this way positive chemotaxis is explained. If, on the other hand, the tension acts in lines of force directed toward the center of the protoplasm, the same mathematical construction shows that the mass will move away from the center of diffusion; thus is explained negative chemotaxis.

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[1] The above quotation from Nägeli is translated from Hertwig, *Die Zelle*, p. 66.

The impossibility of reconciling the movements of the three Infusoria, whose reactions I have described, with any such theory as this is manifest. The theory, though designed expressly to explain the movements of the bacteria and flagellates used in Pfeffer's well-known experiments in chemotaxis, neglects entirely the fact of the differentiation, in those organisms, of axes along which movement takes place, as well as the fact that they move by means of definite organs of locomotion, — flagella, or cilia. To make this abstract scheme fit the concrete motions of the organs of movement would be much more difficult than to invent the scheme.

If chemotaxis acts in the direct manner supposed in such a theory as the above, the organism will of course move directly toward a source of attractive stimulus, directly away from a source of repellent stimulus, and that moreover without regard to the relation of the direction of its axes to the direction of motion. As I have shown in detail for Paramecium (*loc. cit.*), and briefly above for Spirostomum and Stentor, this is by no means true for the organisms studied. On the contrary, the direction of motion has no relation to the position of the source of stimulus, so that we cannot correctly speak of attraction or repulsion at all. The organism reacts *as an individual, not as a substance*, and the nature of the reaction is conditioned by the internal mechanism and the structural differentiations of the body of the organism. The essential distinction insisted upon by Le Dantec (*loc. cit.*) between the reactions of a unicellular organism and those of a metazoan must therefore, for these organisms at least, fall to the ground. It will not do to think of the reactions of these organisms as in any way akin to those of chemical substances.

On the other hand, the reactions are equally distant from the complexity assumed by those who attribute to unicellular organisms most of the psychological powers of higher animals. The reactions of these organisms may best be compared with the working of a machine in which the wheels are geared to turn in but one direction, whatever be the nature of the force that sets them in motion.

DARTMOUTH COLLEGE, HANOVER, N.H.,
February 28, 1899.

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Dr. C. A. Kofoid,
with the regards of H. S. Jennings

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STUDIES ON REACTIONS TO STIMULI IN UNICELLULAR ORGANISMS. IV.—LAWS OF CHEMOTAXIS IN PARAMECIUM.

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STUDIES ON REACTIONS TO STIMULI IN UNICELLULAR ORGANISMS. IV. — LAWS OF CHEMOTAXIS IN PARAMECIUM.[1]

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1. INTRODUCTION.

IN the second paper of this series of Studies,[2] I have given an account of the mechanism of the reactions of Paramecium that requires a revision (for this animal) of the usual conceptions conveyed by the term chemotaxis. It was there shown that Paramecium reacts in essentially the same manner to all effective stimuli, and that the direction of motion in the reaction has no relation to the position of the source of stimulus, so that the animals are not directly attracted or repelled. When the animals are stimulated in any way, they swim backward, turn toward the aboral side, then swim forward over the new path so determined, without regard to the position of the stimulus. The terms positive and negative chemotaxis thus lose their content almost entirely, at least in the construction which is often put upon them. Nevertheless it will be convenient to employ these terms to express the fact that the Paramecia do, in the manner detailed in the paper above referred to, form collections in certain regions (positive taxis), and leave other regions empty (negative taxis). These phenomena retain their biological significance in full, whatever the means by which they are brought about. In view of the mechanism of the reactions, the following definitions may be given. Certain substances in solution set up in a Paramecium random movements, the direction of movement being changed at the time the stimulus begins to act and frequently so long as it continues to act. As a result of these undirected motions the Paramecium is in time, by the laws of chance, carried out of the region of the substance causing them, and prevented from re-entering. Hence toward these substances it may be said to be negatively chemotactic, and the substances may be called repellent. Other substances cause no motor reaction whatever, so that the Paramecia tend to come to rest within them, and these substances are further of such a nature that they throw the Paramecia into a peculiar physiological condition, such that coming in contact with a fluid not containing these substances causes the motor reaction above characterized, so that the animals are returned into the region of the substance causing no motor reaction. Hence if this region is small a dense assemblage of Paramecia may be formed within it. Substances of this character may therefore be said to be attractive, and the Paramecia are positively chemotactic toward them. The terms repellent and attractive, negative and positive chemotaxis, as used in this paper, are then to be understood to have significations in accordance with the above definitions.

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[1] Scientific Results of a Biological Survey of the Great Lakes, directed by Jacob Reighard (Ann Arbor, Mich.), under the auspices of the U. S. Commission of Fish and Fisheries, No. 2. Published by permission of the Hon. George M. Bowers, Fish Commissioner.

[2] The following papers have appeared in the series of Studies of which this is the fourth: I. *Reactions to Chemical, Osmotic, and Mechanical Stimuli in the Ciliate Infusoria*, Journal of physiology, 1897, xxi, pp. 258–321. II. *The Mechanism of the Motor Reactions of Paramecium*, This journal, May, 1899, ii, pp. 311–341. III. *Reactions to Localised Stimuli in Spirostomum and Stentor*, American naturalist, May, 1899, xxxiii, p. 372. These papers will be referred to in the following as I, II, and III, respectively.

2. RELATION OF CHEMOTAXIS TO BENEFIT OR INJURY.

The present paper deals with the nature of the substances thus causing or failing to cause the motor reactions, and with the laws of their action. The first question which will be taken up is in regard to the relation of chemotaxis to the benefit or injury to the organism, caused by the given substance. As shown in II, the effect of the motor reaction is on the whole to carry the organism out of the sphere of action of the substance causing the reaction and to prevent its returning. Is the production of this reaction due to the fact that the substance causing it is injurious to the Paramecia? This is a question of some interest in view of a common theoretical explanation of the origin of chemotactic movements. It is said that through natural selection such individuals as are repelled by injurious substances would survive and perpetuate their kind, while those not repelled by these substances would be destroyed; the converse being true as regards beneficial substances. We might then expect to find that the individuals which now survive have become accurately adjusted in these matters, being repelled by any substance which injures them. Any injury might be conceived to result in the "organic analogue of pain," causing thus motor reactions which take the animal away from the source of injury.

We know already from the first of these Studies that the substances which cause the motor reaction most markedly, and hence give most clearly the phenomena of negative chemotaxis, are chiefly alkalies, while substances in which the Paramecia collect, giving the motor reaction only when they attempt to pass *out* of them, are substances having a weak acid reaction. Thus the distinction between repellent and attractive substances is, to a certain extent at least, based upon purely chemical differences. But it was shown in the same paper that many neutral salts likewise cause the reaction, so that the acid or alkaline character of the solution is not the only determining factor; further, that when acid solutions reach a certain strength, they too cause the same motor reaction as do other substances; it is only weakly acid solutions that are attractive, while stronger acid solutions are repellent. It is a well-known fact that organisms are often thus negatively chemotactic toward substances to which in a weaker solution they are positively chemotactic.

Now, what is the basis of this change of reaction with a change in the strength of solution? The solution of course becomes more injurious as it becomes stronger, and the answer to this question which lies nearest is that the negative chemotaxis of the organisms is due to the injuriousness of the solution. May we say that as soon as an acid solution becomes equally injurious with the repellent alkaline solution, it too becomes repellent? And can we generalize the answer and say that the animals are throughout negatively chemotactic to certain substances in virtue of the fact that these substances are injurious to them?

If the injurious quality of the substance is the determining factor in causing the reactions which result in negative chemotaxis, then evidently two substances which are equally injurious must have equal powers of repelling Paramecia, and if one substance is injurious in a weaker solution than a second, then also must the first substance be repellent in a weaker solution than the second. In other words, the repelling powers of the two substances must be proportional to their injurious effects.

This gives a method of testing the question. We may determine the weakest solutions of two or more substances that repel the Paramecia, and note the ratio of their strength; then determine the weakest solutions of the same substances that will kill the Paramecia in a given time, noting their ratios as before. If the two ratios are approximately the same — that is, if the repellent powers are proportional to the injurious effects — then the evidence is in favor of the proposition that the production of the motor reaction resulting in repulsion depends upon the injurious effect of the substances; if the ratios are not the same, then the relation between repellent power and injuriousness is not one of direct dependence.

I have compared in this way a considerable number of substances. The repellent powers of different chemicals were tested in the manner described in I and II for studying chemotaxis, — by introducing with a pipette drawn to a capillary point a drop of the substance beneath the cover-glass of a slide of Paramecia. The substances to be tested were dissolved in proper proportions in distilled water. In distilled water alone the Paramecia form collections (as shown in I), so that the repellent powers of the solutions are due wholly to the dissolved substance. For finding approximately the weakest solution of a substance that causes repulsion, it is convenient to proceed as follows: A series of covered watch-glasses are filled with solutions of the given substance, each successive watch-glass with a solution one half or one fourth as strong as that in the preceding one. Thus we shall have perhaps the following solutions: $\frac{1}{5}$, $\frac{1}{20}$, $\frac{1}{80}$, $\frac{1}{320}$, and $\frac{1}{1280}$ per cent, etc. The Paramecia are placed on a slide beneath a long cover-glass supported at the ends by bits of glass tubing. Now with the capillary pipette drops of solutions of different strengths are introduced beneath the four corners of the cover-glass, — under one corner a drop of $\frac{1}{5}$, under the next $\frac{1}{20}$, then $\frac{1}{80}$, and $\frac{1}{320}$ per cent. We shall probably find that some of the drops remain empty, while others are quickly filled with Paramecia. Suppose that the $\frac{1}{5}$, $\frac{1}{20}$, and $\frac{1}{80}$ per cent remain empty, while the $\frac{1}{320}$ per cent is quickly filled. It is thereupon evident that the weakest solution which repels the Paramecia lies somewhere between $\frac{1}{80}$ and $\frac{1}{320}$ per cent. By making proper intermediate grades between these two, approximately the weakest solution that repels the Paramecia can usually be determined very expeditiously. All the precautions insisted upon in I as necessary for carrying on such experiments were of course rigorously observed.

I will present in detail the results in two typical cases, then summarize my results on the sixty-five substances tested, in the form of a table.

For a first case we may take two chemically related substances, toward both of which the Paramecia show positive chemotaxis when the solutions are very weak. Chromic acid and potassium bichromate fulfil these conditions. These substances both have an acid reaction, due to the same component in each case, and the Paramecia gather in weak solutions of both of them. In stronger solutions both cause the motor reaction which results in repulsion. Is this due in each case to the injurious effect of the stronger solutions?

For potassium bichromate the weakest solution that repels the animals is about $\frac{1}{20}$ per cent. For chromic acid the weakest solution which sets the motor reaction in operation is about $\frac{1}{150}$ per cent. The ratio of repellent powers is therefore 7.5 to 1 in favor of the chromic acid.

The injurious effects of the solutions are now tested as follows: Watch-glasses with solutions of different strength are arranged in series, as above described, and into each a drop of water swarming with Paramecia is introduced and quickly mixed. The solutions are made in such a way that after the introduction of the water containing Paramecia they are of the recorded strength. The weakest solution in which all the Paramecia are killed in one minute is thus determined for the two substances.

For the potassium bichromate it is found in this way that the weakest solution which kills the Paramecia in one minute is a one per cent solution, — that is, a solution twenty times as strong as the weakest solution that repels them. In the chromic acid they die somewhat more quickly in a $\frac{1}{150}$ per cent solution, — a solution of the *same* strength as the weakest which causes repulsion. The ratio of the injurious effects of the two substances is therefore as 150 to 1 in favor of the chromic acid.

Comparing the two ratios, we find that they are strikingly unequal. While chromic acid has but 7½ times the repellent power of potassium bichromate, it is 150 times as injurious. Potassium bichromate repels in a strength $\frac{1}{20}$ of that which is severely injurious; the chromic acid does not repel until it has reached a strength which is already destructive. In the latter case the repellent power is evidently due directly to the injurious effects; repellent power and injurious effects appear at the same point as we pass from weaker to stronger solutions. If the conduct of the Paramecia is observed under the microscope as they swim backward after coming in contact with a drop of $\frac{1}{150}$ per cent chromic acid, it is at once evident that they are decidedly injured, and frequently they die after swimming backward a little distance. The motor reaction is thus not set in operation by the chromic acid until the Paramecia are already injured and it is too late to save them. When such a drop is introduced beneath the cover-glass, the Paramecia are therefore killed by scores and the drop is soon surrounded by a ring of the dead animals. If a drop of a solution somewhat weaker than $\frac{1}{150}$ per cent is introduced, the Paramecia enter it directly, forming a dense gathering, and after a time it may be observed that many individuals of the group are dead or severely injured. In the case of the potassium bichromate, on the other hand, the Paramecia are never injured whatever the strength of solution used; they gather in a ring about a drop of strong solution, but do not enter it deeply enough to be injured.

For these two substances then the test results negatively; potassium bichromate has twenty times the repellent power it should have if repellent power depends alone on the injuriousness of the solutions.

We may next test two substances which do not attract the Paramecia in any strength, and which are not injurious in virtue of their chemical properties at all, but only as a result of their osmotic pressure. As such substances we may select sodium chloride and cane sugar. Repeated comparative tests with isotonic solutions of these two substances indicate that neither is immediately injurious to the Paramecia except through osmotic pressure; strong isotonic solutions of the two substances kill the Paramecia in approximately the same period of time. Thus if Paramecia are introduced at the same time into 1 per cent sodium chloride solution and 9.64 per cent cane sugar (which is isotonic with 1 per cent solution of sodium chloride), in both cases nearly all the individuals are found to be dead twenty minutes after they were introduced, and in neither solution does the proportion of dead markedly preponderate. This result for Paramecium differs from that gained by True[1] for Spirogyra; in the case of Spirogyra sodium chloride was found to have a distinct toxic effect in addition to its osmotic properties.

The weakest solution of sodium chloride which repels the Paramecia is from $\frac{1}{10}$ to $\frac{1}{20}$ per cent. The weakest solution of cane sugar which sets the motor reaction clearly in operation is 10 per cent. Taking the repellent solution of sodium chloride as $\frac{1}{10}$ per cent, the repellent power of sodium chloride is to that of cane sugar as 10 to $\frac{1}{10}$, or as 100 to 1. On the other hand, as stated above, 1 per cent sodium chloride has the same injurious effect as 10 per cent cane sugar, so that the injurious effects of sodium chloride are to those of cane sugar as 10 to 1. The repellent power of the cane sugar, like that of the chromic acid, is plainly due to its injurious properties. The motor reaction is not set in operation until plasmolysis has begun, as shown by the evident shrinkage of the bodies of the animals, and it is usually too late at this time to save them from destruction. If a drop of strong sugar solution is introduced into a slide of Paramecia, it soon becomes filled with the dead Protozoa. On the other hand a drop of sodium chloride is not destructive whatever its strength, since the motor reaction is set in operation long before the Paramecia have entered deeply enough to be injured. Sodium chloride has, as shown by the ratios given above, ten times the repellent power that it should have if repellent power depends entirely upon injurious effects.

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[1] TRUE: Botanical gazette, 1898, xxvi, p. 407.

A large number of compounds, chiefly inorganic, were tested as to the relation between repellent power and injurious effects, and it was found that the cases given above are typical for all. We can divide chemical substances as regards this matter into two classes, as shown in the following table. In the first column may be placed together substances in which, as in potassium bichromate and sodium chloride, the repellent power is great in proportion to the injurious effects. These substances all set the motor reaction in operation long before any injury has taken place, so that the Paramecia are not harmed in the least when a drop of one of them is introduced into a slide preparation of the infusoria. In the other column may be placed substances in which, as in chromic acid and cane sugar, the repellent power is slight in relation to the injurious effects ; the motor reaction in all these substances is set in operation only when the Paramecia are already injured and it is too late to save them. A drop of any one of these substances introduced beneath the coverglass of a slide preparation of Paramecia proves exceedingly destructive.

We may for convenience classify the substances in the table also into those which are attractive to the animals in weak solutions (A), and those which are repellent in all effective solutions (B).

TABLE I.

1. Repellent power strong in proportion to injurious effects: chemotaxis protective.	2. Repellent power very weak in proportion to injurious effects: chemotaxis not protective.
(A. *Attractive Substances.*)	
Sodium fluoride, Potassium bichromate, Ammonium bichromate, Potassium ferricyanide. (KBr), (KCl), (RuCl), (CsCl).[1]	HF, HCl, HBr, HI, H_2SO_4, HNO_3, Acetic Acid, Tannic Acid, Picric Acid, Chromic Acid, Potassium fluoride, Potassium permanganate, Ammonia alum, Ammonio-ferric alum, Chrome alum, Potash alum, $CuSO_4$, $CuCl_2$, $Cu(C_2H_3O_2)_2$, $ZnCl_2$, $HgCl_2$, $AlCl_3$.
(B. *Repellent Substances.*)	
LiCl, NaCl, (KCl), (RuCl), (CsCl),[1] LiBr, NaBr, (KBr), RuBr, LiI, NaI, KI, RuI, Li_2CO_3, Na_2CO_3, K_2CO_3, $LiNO_3$, $NaNO_3$, KNO_3, NaOH, KOH; $KBrO_3$, NH_4F, NH_4Cl, NH_4Br, NH_4I, $CaCl_2$, $SrCl_2$, $BaCl_2$, $Ca(NO_3)_2$, $Sr(NO_3)_2$, $Ba(NO_3)_2$.	Cane sugar, Lactose, Maltose, Dextrose, Mannite, Glycerin, Urea.

From this table and the foregoing discussion it is evident that the question proposed is to be answered in the negative. The injuriousness of a substance is not the determining factor in its repellent power, since the repellent power of different substances is not at all proportional to the injuriousness of their solutions. In the substances of the second column, such power as they have to set in operation the motor reaction of Paramecium is indeed evidently due directly to the injuries caused, though this power is nothing like so precise and strong as in the substances of column 1, not being sufficient even to prevent the Paramecia from entering and being destroyed.

The fact that the injuriousness of a solution is not the chief determining factor in its repellent power seems to indicate that the common theory, according to which the reactions of organisms have been fixed by natural selection through the destruction of the individuals not repelled by injurious substances, does not contain the whole truth.

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[1] In the case of the substances enclosed in parentheses, the reactions of Paramecia from different regions varied, as described in a subsequent part of this paper.

Probably many of the substances experimented with have never been met by the Paramecia in the course of their evolution, and their reaction toward these must be determined by other laws. Possibly their reactions are adjusted by natural selection to some small number of substances, while to other substances they react by analogy as it were, — by the similarity of their effects to those of the substances to which the reaction has been adjusted by natural selection. Thus it may be that repulsion for some alkali plays an important part in the biology of the animals, and that the reaction toward other substances of this sort is due to their similarity to the primary one. We know that to the tendency to collect in weak solutions of carbon dioxide is due the apparently gregarious habit of the Paramecia, and this probably serves a useful purpose somewhere in the life of the animals. It may be that the tendency to gather in other acid solutions, even such destructive ones as sulphuric acid and copper sulphate, is a sort of reflection of this tendency to collect in carbonic acid, and that natural selection has had no opportunity to correct this tendency directed toward injurious substances. That there is much opportunity for natural selection to act is clear when one tests any chemical substance by bringing a drop of it among a large number of Paramecia. There is always a number of individuals who do not give the typical reaction, some being much more and some much less sensitive than the majority.

Another consideration of importance is the following: It may not be at all the *direct* effects of a substance on the Paramecia, through which natural selection has adjusted the reaction toward that substance. Thus it may be that in solutions of a certain nature, harmless in themselves, the Bacteria which form the food of the Paramecia are never found; natural selection would then act by starving such Paramecia as were not repelled by these solutions.

3. RELATION OF CHEMOTAXIS TO CHEMICAL COMPOSITION.

If the injuriousness of substances is not the determining factor in their repellent power, what *is* the determining factor? An examination of the table above given brings out the following facts. All the substances in which the repellent power is relatively great contain a large proportion of some metal of either the alkali group (Li, Na, K, Ru, Cs, or NH_4) or of the earth alkali group (Ca, Sr, Ba). This is true of all, whether having an acid, a neutral, or an alkaline reaction. On the other hand most of the substances in the second column contain none of these elements. It is true that certain of these compounds, as potassium fluoride, potassium permanganate, and the alums do contain members of the alkali group. But in potassium fluoride the qualities of the compound are due almost entirely to the fluorine component, as is shown by the fact that this salt, like hydrofluoric acid itself, attacks glass energetically. In potassium permanganate and the alums the proportion of alkali metal is small, as is shown by their formulas. Thus the formula for potash alum is $KAl(SO_4)_2, + 12\ H_2O$, so that in the molecule composed of forty-eight atoms but one of these is K; the properties of these compounds are very little due to the alkali component. The proportion of alkali metal is very much larger in the substances of the first column. This dependence upon the presence and proportion of an alkali metal is in practice so striking that in working with salts whose action on the Paramecia is not yet known, it is usually possible to predict with certainty from the chemical formula into which column they will, upon experiment, be found to fall.

The results on these sixty-five compounds appear then to justify the following conclusion: Compounds which contain a large proportion of one of the alkali or earth alkali metals set in operation the motor reaction of Paramecium even when exceedingly weak; their repellent power is therefore great in proportion to their injurious effects, and the infusoria are not endangered by the presence on the slide where they are swimming of a drop of a solution of these compounds. Substances which do not contain a large proportion of one of these metals as a rule set in operation the motor reaction of Paramecium only when the animals are already injured; their repellent powers are very weak in proportion to their injurious effects, and a drop of one of them introduced into a preparation of Paramecia is very destructive to the latter.

The fact, however, that the injurious properties of a substance on reaching a certain intensity of action do produce the motor reaction is worthy of special notice; in all the substances in the second column the reaction is manifestly due directly to the injuries inflicted, without regard to the nature of the substance. Paramecium is not sufficiently sensitive to injuries to make the reaction thus produced of any protective value, at least under experimental conditions. But it may easily be conceived that this sensitiveness might be so increased that the reaction would be of the greatest importance for preserving the animals; it is probable that in many organisms this condition is realized. We seem to have in Paramecium the very lowest step in the production of reactions due to the "organic analogue of pain" (to use a phrase employed by J. Mark Baldwin), — a step so low that the power of thus reacting seems to have no value for the life of the organisms.

The chief determining factor in the repellent power of solutions is then, for Paramecium, of a chemical nature, with little relation to the comparative injuriousness of the solutions.

The conclusion above drawn as to the repellent power of the alkali metals is reinforced and extended by certain results gained in a study of the compounds of these metals with the halogens, — compounds of lithium, sodium, potassium, rubidium, and cæsium on the one hand, with fluorine, chlorine, bromine, and iodine on the other. These form a definitely circumscribed group of compounds, the characteristics of which, as is well known, vary in many respects with the molecular weights of the elements composing them. A qualitative and quantitative study of the reactions of the Paramecia toward these compounds was begun at Put-in-Bay Island, Lake Erie, during the summer of 1898, in the hope that such a study would throw light on the essential nature of chemotaxis. The work was not finished at Put-in-Bay, and on taking it up again at Hanover, N. H., it was found that the Paramecia here gave in part different reactions from those studied at Put-in-Bay. The differences are of such a nature as to be not unintelligible, yet it will be necessary to consider separately the results obtained at the two places.

The elements belonging to the group of halogens may be arranged in a series, beginning with that having the least atomic weight and proceeding to that which has the greatest atomic weight, as follows: fluorine (at. wt. 19), chlorine (at. wt. 35), bromine (at. wt. 80) iodine (at. wt. 127). As is well known, this is also the order of the chemical activity of these elements, the lightest being most active. In the same way the alkali metals may be arranged in a series beginning with the lightest, as follows: lithium (at. wt. 7), sodium (at. wt. 23), potassium (at. wt. 39), rubidium (at. wt. 85), and cæsium (at. wt. 133). This series also gives in a general way the order of chemical activity of the elements, the lighter ones being more active.

The compounds of these two sets of elements may be arranged in a table in such a way as to give the halogen series from above downward, the alkali series from left to right. In the ensuing table, arranged in this manner, are placed only the compounds which I was able to procure and use for studying the reactions of the Paramecia at Put-in-Bay. For convenience the hydroxides of the metals and the acids of the halogens are brought into the table, — the former constituting the lower row, the latter the right-hand column.

TABLE II.

	Li(7)	Na(23)	K(39)	Ru(85)	Cs(132)	
F(19)	....	$\underline{NaF}$	$\underline{KF}$	....	....	$\underline{HF}$
Cl(35) . . .	LiCl	NaCl	$\underline{KCl}$	$\underline{RuCl}$	$\underline{CsCl}$	$\underline{HCl}$
Br(80) . . .	LiBr	NaBr	$\underline{KBr}$	....	....	$\underline{HBr}$
I(127) . . .	LiI	LiI	KI	....	....	$\underline{HI}$
		NaOH	KOH			

In this table, as will be observed, the compounds of the lighter (and more active) metals are placed to the left; the compounds of the lighter (and more active) halogens in the upper row. In the right-hand upper part of the table are given therefore compounds of light, active halogens with heavy, inactive metals; in these the influence of the halogens might be expected to predominate. In the left-hand lower corner are given compounds of light, active metals with heavy, inactive halogens; in these the metal component might be expected to predominate.

Now some of these substances repel the Paramecia in any effective concentration; that is, they produce the motor reaction which results in carrying the Paramecia outside of their influence; these we may call the repellent substances. Others when weak do not cause the motor reaction when the Paramecia enter them, but when the animals attempt to swim *out* of the drop into the surrounding water the motor reaction occurs, and the Paramecia return into the drop. They thus form collections either within such drops or surrounding them in a close ring, — in the manner described in I, p. 269. These we may call attractive substances.

All the attractive compounds in the above table are underscored; those not underscored are repellent. It will be seen, first, that all the acids or hydrogen compounds of the halogens are attractive, the hydroxides, or hydroxyl compounds of the metals repellent. Further, that all the compounds of the lightest, most active halogen (fluorine) are attractive. Of the next lightest, chlorine, three salts are attractive and two repellent. Of the heaviest, most inactive halogen, iodine, all the salts studied are repellent. Of the lightest, most active metal, lithium, all the salts tested are repellent, and the number of repellent salts decreases as we pass toward the heavier metals. Thus the compounds of light, active metals with heavy, inactive halogens are repellent; those of light, active halogens with heavy, inactive metals are attractive. A diagonal line can therefore be drawn, as shown in the table, from the upper left hand to the lower right hand, separating all the attractive substances on the right from all the purely repellent substances on the left.

It thus appears that the Paramecia react to these compounds with relation to the elements of which they are composed. The metal components tend to repel the Paramecia; the halogen components to attract them. The acids of the halogens, in which the halogen may be presumed to give character to the compound almost alone, do not repel except in such solutions as to be immediately destructive, as previously discussed. The hydroxides, or bases, in which the metals give the character to the compound, are repellent in any solution strong enough to produce any effect whatsoever. Those compounds which are partly metal and partly halogen are attractive or repellent in accordance with whichever one is the predominant element.

The same conclusion is reached by a study of the exact method of behavior of the Paramecia toward the substances above classed as attractive. Toward these compounds the infusoria are as a rule *both attracted and repelled.* As just stated the acids are purely attractive, the bases purely repellent. Toward potassium fluoride, in which the halogen component is so active as to attack glass even in this salt, the animals react as toward a pure acid. Toward sodium fluoride, in which the fluorine is so bound by the sodium that the two produce a comparatively inactive salt, the Paramecia are both strongly attracted and strongly repelled. When a drop of one per cent sodium fluoride solution, for example, is introduced into a slide of Paramecia, it will soon be found that the animals have gathered in a dense ring about the drop, leaving its centre entirely free. In this ring they swim back and forth, giving the motor reaction whenever they come to the inner side of the ring, where the stronger sodium fluoride is, or when they come to the outer side of the ring, where pure water is. Sodium fluoride thus gives a striking example of apparently positive and negative chemotaxis to the same substance. Of compounds outside the group at present under consideration, potassium and ammonium bichromate and potassium ferricyanide show precisely the same phenomena.

The other salts classified in the table as attractive show the same phenomena as sodium fluoride, except that the attraction is less strong and the repulsion stronger. The Paramecia gather in a narrow ring about them, and this ring contains at a given time comparatively few individuals, though enough to make the ring evident as a rather sharply defined white line around the drop. If the individuals constituting this ring are closely observed, it becomes evident that the motor reaction is set strongly in operation when they come to the inner boundary of the ring, where the stronger solution of the salt is found, and much less strongly when they come to the outer boundary, against the ordinary water. Thus many individuals remain in the ring but a short time, finally crossing the outer boundary and escaping, so that the ring does not increase continually in numbers and size, as it does in the case of sodium fluoride; it consists of changing individuals, each one being detained usually but a short time. In some of the salts the ring thus formed is almost evanescent: for a short time it will be distinct, then almost disappear, then reappear again. Finally, about the salts which are not underscored in the table no ring of Paramecia is ever formed.

This formation of a ring of greater or less extent about solutions of various salts is a very curious phenomenon. The Paramecia are evidently thrown into such a condition by a very weak solution of the substance that the contact with the surrounding water now acts as a shock or stimulus to set the motor reaction in operation, — while this motor reaction is also induced by a stronger solution of the same salt, such as is found nearer the centre of the drop. In view of the facts adduced, it seems apparent that the shock on reaching again the outer water is due to the physiological effect of the halogen component, as this shock alone is produced in compounds in which, as in acids and potassium fluoride, the halogen is the element that chiefly gives character to the solution. On the other hand, the stimulus due to entering the salt itself is a result of the metal component, since this stimulus alone is produced in those compounds in which, as in hydroxides (and carbonates) the characteristics of the compound are due chiefly to the metal.

The above results were obtained at Put-in-Bay in a series of about 1000 tests of the substances in the table. The compounds used were tested many times, dissolved both in distilled water and in the same water in which the Paramecia were found, with every possible precaution to secure accuracy, and with uniform results as given above. But on continuing the series of experiments at Hanover, N. H., with the intention of filling the gaps in the table as above given, different results were obtained with the Paramecia of this region for certain of the salts. Toward KCl, KBr, RuCl, and CsCl the Paramecia of Hanover show only repulsion, without any sign of the formation of a ring about them, which was so evident at Put-in-Bay. Toward the NaF and KF on the other hand they react exactly as did those at Put-in-Bay, forming a ring about the NaF and reacting toward the KF as toward an acid. Toward all other members of the above table the Hanover Paramecia likewise react exactly as did those at Put-in-Bay. This variation is evidently not of a character to modify in any way the conclusions to be drawn. The Paramecia at Hanover simply fail to show a very faint reaction given by those from Lake Erie in the presence of certain substances; they seem therefore merely slightly less sensitive; in other respects the reactions are similar in all the Paramecia studied. The Paramecia at Put-in-Bay were cultivated in lake water containing decaying water plants, chiefly Nuphar, Naias, and Ceratophyllum. At Hanover the Paramecia came from cultures of hay, in water from small ponds of the neighborhood. Possibly the difference in the culture medium has something to do with the difference of reaction in the Paramecia of the two regions.

The reactions toward certain other substances may be stated briefly. Toward the carbonates of lithium, sodium, and potassium the reaction is the same as toward the hydroxides of these metals. Also toward the nitrates of these metals and toward the chlorides and nitrates of calcium, strontium, and barium the reaction is of the nature of repulsion alone, as toward the iodides, sodium chloride, etc., given in the table. Toward salts of the heavy metals, as cupric chloride, cupric sulphate, mercuric chloride, zinc chloride, aluminium chloride, the Paramecia are attracted, as toward acids.

From the facts given in the above table and discussion the following conclusions may be deduced: —

1. The base-forming elements of the alkali and earth alkali groups when present in a compound tend to produce the motor reaction in Paramecia whenever the animals come in contact with these compounds, even in very dilute solutions. This may be put in terms of the modern theory as follows: the kations of the alkali and earth alkali groups have a strong tendency to induce the motor reaction in Paramecia. As a consequence these infusoria are kept from entering such solutions even when the solutions are very weak.

2. The ions of the acid-forming elements, such as the halogens, and in fact the anions of *all* acids, do not themselves tend to cause the motor reaction in Paramecia, except when present in such strength as to be immediately destructive. On the contrary, they tend to throw the Paramecia into such a physiological condition that contact with any solution containing no anions produces the motor reaction. As a consequence, Paramecia tend to congregate in regions where it is mainly the anions that give character to the solution.

The fact that the same result as for acids is obtained for salts in which the anion is much the most powerful component, as in potassium fluoride, shows of course that the results with acids are not due to the hydrogen ion of the acids.

3. Compounds that contain both kations of the alkali elements, and anions, may give a combination of these results, — a tendency to cause the motor reaction (with its reversal of cilia) both when the animals enter the solution (due to contact with the kations), and when they leave the solution (effect of the anions). In this case the Paramecia collect in a ring about a drop of the compound.

4. Salts that contain the relatively inactive kations of one of the heavy metals, as aluminium, copper, zinc, mercury, produce an effect due only to the anion, hence their effect is like that of an acid.

These conclusions in regard to the different effects of the anions and kations on the motor reactions of Paramecium certainly seem to invite a discussion of the relation of the activities of the ions during the passage of an electric current through a solution to the movements of the Paramecia under the same conditions. As is well known, when a continued current is passed through the water containing Paramecia, they all swim with one accord to the kathode, — thus in the same direction as that in which the metallic ions are moving. It is difficult to resist the impression that there must be some such relation between the movements of these ions and the movements of the organisms as has been suggested by Loeb and Budgett.[1] Yet a discussion of this matter will hardly lead to valuable results until it can be accompanied by a careful re-examination of the phenomena of electrotaxis, with especial reference to the relation of the electrotactic movements to the general motor reaction of Paramecium described in the second of these Studies. We possess already a detailed account of the movements of the cilia in electrotaxis, due to Ludloff,[2] but these observations, as well as my own observations on electrotaxis, published in I, were made before the general plan of the motor reactions of Paramecium was known, so that a re-examination might lead to the discovery of facts of fundamental significance.

4. RELATIVE REPELLENT POWER OF RELATED SUBSTANCES.

In the first part of this paper I have shown that with relation to their action upon Paramecium, chemical substances are divisible into two classes, in one of which the repellent power is very great in proportion to the injurious effects, while in the other class such slight repellent power as exists is due directly to injuries caused. In this latter class a quantitative study of the relative repellent powers of the different members shows of course that the power of setting in operation the motor reaction of Paramecium is in a general way proportional to the injuriousness of the solutions. But to what factor are the relative repellent powers among themselves in the members of the *first* group due? I have already shown that the relative repellent power of members of the first group, as compared with that of members of the second group, is due to the presence in preponderating amounts of some metal of the alkali or earth alkali series. But this leaves untouched the question of the relative repellent powers of members of the first group among themselves. For example, will sodium chloride be more or less repellent than potassium chloride, and why?

On the question of the relative repellent powers of different members of this group much labor was spent, with rather unsatisfactory results. This is due to the fact that the different compounds have specifically different ways of affecting the Paramecia, some modifying the general motor reaction in one way, some in another, — although never changing the essential character of the reaction. This is a matter worthy of special study. Chemotaxis is a complex phenomenon, the motion to or from a substance being the indirect resultant of the specific physiological effects of this substance upon the different phases of the motor reaction. These specific physiological effects are therefore the primary phenomena. This fact was not recognized at the time my work was done, so that full records were not made on these points. On a few substances observations were made, which I will give here as examples. As shown in II, the typical motor reaction is as follows: (1) the cilia are reversed, causing the Paramecia to swim backward; (2) the aboral cilia of the anterior half of the body strike transversely toward the oral side, turning the animal toward the aboral side; (3) the cilia all strike backward as at first, carrying the animal forward over a path which lies at an angle to the original path. This reaction is given in characteristic form when the animal comes in contact with a weak alkali; all the parts are fairly though not excessively pronounced. On coming in contact with a strong acid solution, the Paramecia dart furiously backward a long distance. Then they turn and dart furiously forward, so that they often thus rush directly into the drop of acid, and perish. If their course after turning, however, tends to carry them away from the acid, when they strike the water into which no acid has diffused they react again by reversing and turning, — showing that the acid has put them into a peculiar physiological condition. Some acid substances cause the reaction with certain of its typical features nearly or quite suppressed. Thus when the animals swimming forward come in contact with diffusing mercuric chloride, they dart straight backward, the anterior end being curved a little to one side; at the same time the body begins to swell. Then they start straight forward again, the turning usually being nearly or quite omitted. Paramecia which come in contact with a drop of $\frac{2}{5}$ per cent calcium chloride solution while swimming forward, pass in without hesitation, but are excited and the forward course is hurried, so that they soon pass out. They thus never remain quiet in the calcium chloride, and as a result it remains nearly empty, the Paramecia gradually settling down elsewhere. Yet one cannot say that the calcium chloride repels the animals; it merely excites them, without causing even a change in the direction of motion. Paramecia introduced directly into 2 per cent barium nitrate solution are paralyzed instantly. But in three or four minutes they revive, and swim slowly about. In 2 per cent calcium nitrate solution, on the other hand, they are at once violently excited, swimming forward and backward swiftly. In solution of strontium nitrate they are likewise excited, but much less so. In 1 per cent sodium bromide and sodium iodide they first dart backward, then turn, then swim forward, then backward again, — repeating the entire typical motor reaction many times. By 1 per cent potassium iodide each part of the motor reaction is almost indefinitely prolonged; they swim backward perhaps ten minutes at a time, then they turn toward the aboral side, and *keep on turning*, whirling thus on the short axis for twenty minutes or more. In 10 per cent solution of cane sugar they at first give no reaction; gradually as plasmolysis occurs they begin to swim backward, then turn, then swim forward, repeating this, as in sodium bromide solution. It will readily be seen in view of these peculiarities in reaction, that it is not always possible to classify the conduct of the Paramecia at once into the two categories of attraction and repulsion.

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[1] LOEB and BUDGETT: Archiv f. d. ges. Physiol., 1897, lxv, p. 518.

[2] LUDLOFF: *Ibid.*, 1895, lix, p. 525.

Moreover, toward many compounds there exist great individual differences among the Paramecia as to the reaction given; at times a few individuals will swim directly into a solution, while the rest leave it empty. There is thus no absolute criterion for determining at what strength the repulsion of a solution really ceases. Moreover, the sensitiveness of the Paramecia to solutions of different strength of a given substance seems to vary according to something like Weber's law, — so that the least observable differences in reaction are between that due to a solution of given strength, and that due to a solution of twice this strength. It is often difficult to determine, for example, whether the repellent power of a substance ceases at $\frac{1}{40}$ or $\frac{1}{80}$ per cent; but upon this decision may depend our conclusion as to which of two substances shows the greater repellent power.

The following results upon certain salts are, however, based upon a very large number of experiments, and so far as they go, may be given with confidence. The method of experiment was that already described, the two substances being compared always under exactly similar conditions in all respects, by introducing a drop of each beneath the cover-glass of the same slide of Paramecia. A special study was made in this way of the halogen salts of the alkali metals, — the chlorides, bromides, and iodides of lithium, sodium, potassium, rubidium, and ammonium, together with the chloride of cæsium. This study was made at Hanover, and therefore upon Paramecia that were repelled by all these salts, so that the repulsion was not confused with any accompanying attraction.

As explained in connection with certain similar results given in I, it is not possible to give a figure which shall represent in a general way the weakest concentration of any one of these substances that causes repulsion in the Paramecia, on account of the very great variation in the reaction of Paramecia from different cultures and under different conditions. For example, on testing Paramecia from two different cultures at the same time, it was found that those from one culture were decidedly sensitive to lithium chloride in a $\frac{1}{40}$ per cent solution, leaving a drop of this strength empty for a long time, while those from the other culture were quite indifferent to the same chemical even in a strength sixteen times as great, swimming in and out of a drop of $\frac{2}{5}$ per cent solution of lithium chloride without reaction. But the *relative* sensitiveness of Paramecia to two or more different chemicals seems to remain fairly constant, so that the order of sensitiveness to different chemicals can be determined. I give therefore the order of sensitiveness to the different metal compounds of each halogen, — the test of sensitiveness being the relative strength of solution of the different substances that causes repulsion.

For the chlorides the order is as follows, beginning with the most repellent: LiCl, KCl, NaCl, RuCl, CsCl. That is, the repellent power decreases as the atomic weight of the metal component increases, except that the sodium compound is moved down one place, being less repellent than the compound of the heavier potassium. The weakest repellent solution varies from about $\frac{1}{40}$ per cent in the lithium chloride to about $\frac{1}{2}$ per cent in the chloride of cæsium.

For the bromides the order is, beginning with the most repellent, LiBr, KBr, NaBr, RuBr; for the iodides LiI, KI, NaI, RuI. In these two sets of salts the order is thus the same as for the chlorides. In general, therefore, the repellent powers of the halogen salts of the alkali metals varies inversely with the atomic weight of the metal components, except that in each case the sodium compound is less repellent than the heavier potassium compound. The ammonium salts were found in each case to possess almost exactly the same repellent power as the corresponding salts of potassium.

Neglecting for the moment the inverted order in the potassium and sodium salts, two causes suggest themselves for the inverse proportion between the repellent power and atomic weights of the elements. The halogen component remaining the same throughout a given series, the molecular weights of the salts of course increase with the atomic weights of the metal components. Hence the less repellent compounds have the greater molecular weight, so that a solution of given strength by weight of one of these salts contains fewer molecules than does the corresponding solution of one of the lighter salts. Supposing the molecules to have throughout the same repellent power, the solution of the lighter salt, containing more molecules, will of course be more repellent. A second cause may be the usual greater chemical activity of lighter elements. If both these causes are efficient factors, they of course reinforce each other in the series above given. The reason for the anomalous position of the sodium salts, as weaker than the potassium salts, does not appear.

On comparing the series of the different halogen salts of a given metal, as NaCl, NaBr, NaI, I have found it impossible, in spite of much labor directed upon this point, to determine satisfactorily the relative repellent powers. Apparently the three salts have almost exactly equal repellent powers, and many sets of experiments upon the corresponding series for each of the four metals, lithium, sodium, potassium, and rubidium, have not made it possible to differentiate them. In every case the repulsion was very nearly equal for all, and the slight differences observed in one set of experiments did not hold for the next. It is evident that the two factors given above as possibly reinforcing each other in causing the greater repellent power in the lighter salts of the metal series of a single halogen, would, in the halogen series of a single metal, work in opposite directions. The greater number of molecules in the lighter compounds (as of chlorine) would tend to produce greater repulsion. But the greater chemical activity of the lighter chlorine component would tend to neutralize the effect of the metal component, to which alone the repulsion is due. Possibly it is to this interference that the approximately equal repellent power of the chlorides, bromides, and iodides of a given alkali metal is due.

Much time was spent on a similar quantitative study of the nitrates of the alkali metals and of the earth alkali metals, but without clearly defined results. The different nitrates have specifically different effects on the Paramecia; the repellent power is the resultant of several factors, any one of which may vary independently of the others. Thus the phenomena differ in the different salts, and there is no certain criterion for comparing the repellent powers of two salts having different specific physiological effects.

The above somewhat fragmentary and partially negative results of quantitative studies are given, not only because the data obtained are of themselves of some value, but also because it is of importance to show which lines of investigation promise to be fruitful and which do not. This method of study has thrown chiefly a negative light upon chemotaxis, — showing what it is *not*. The evidence that chemotaxis is not a direct simple phenomenon, due to simple forces of attraction and repulsion, is in itself of importance.

The foregoing study of laws of chemotaxis in Paramecium, while it is believed to contain some results that are new and significant, is not put forth as in any sense exhaustive. Certain laws came forth clearly, while certain lines of investigation proved comparatively unfruitful; these I have set forth for the positive value of the one and the negative value of the other. The work has been done chiefly on inorganic compounds; almost the whole field of organic compounds remains to be examined. Certain facts in the reactions toward inorganic substances remain at present isolated, not falling under any of the general laws above given; thus certain experiments seem to show that there is a strong repulsion exhibited toward iodine, bromine, and chlorine when uncombined, — though in the ionic condition these elements are purely attractive. The effect of metals other than the alkalies and alkaline earths, when uncombined with a strong acid, is of interest, and has not been determined. Finally, an examination of the specific physiological effects of chemical compounds on the different parts of the motor reaction, as well as on the substance and the general functions of the animals, would be of fundamental value. Such a study would not be directed purely upon one aspect, as upon repulsion and attraction, which are really the resultant of complex factors, but should have in view any change produced by the substances studied

SUMMARY AND CONCLUSIONS.

In the introduction to the first of the Studies of which this is the fourth, the program for work was given as the investigation of the life activities of a single unicellular organism as completely as possible, in the hope that when such a study should have been carried to some degree of completeness, it should furnish a key to the understanding of the activities of other organisms, and should throw light on the essential nature of chemotaxis and similar phenomena. The investigation of the activities of Paramecium thus begun is far from being complete, yet I believe it may be said that the results thus far gained have already justified the plan of work. The mechanism of attractions and repulsions has been made out, and the third paper of the series shows that this furnishes at once a key to the understanding of the activities of certain other infusoria (Spirostomum and Stentor). Studies now in progress show that it throws equal light upon the activities of other, more distantly related, organisms. All the Studies thus far published have dealt more or less with chemotaxis, and in concluding the present paper upon that subject, it will be well to summarize in the form of brief statements the important conclusions in regard to the phenomena which have gone under this name, drawing upon the previous Studies as well as the present one for this purpose and emphasizing some important points which have not been emphasized elsewhere.

1. The motor reaction by means of which chemotaxis is brought about is identical with that produced by mechanical shock or any other stimulus, so that chemotaxis is not an activity differing *in kind* from others. This motor reaction is as follows: the animal swims backward, turns toward one side which is structurally defined (the aboral in Paramecium), then swims forward.

2. This motor reaction is the same, whatever the position of the chemical or other stimulus, or the part of the body of the animal that it acts upon, so that the same chemical which when acting on the anterior end of the animal causes it to move away, causes it when acting upon the posterior end to approach. The direction of motion has no relation to the localization of the stimulus. The usual relation of the direction of motion to the position of the stimulus depends upon the fact that owing to the forward motion, stimulation almost universally occurs at the anterior end.

3. Negative chemotaxis or repulsion toward any substance is due merely to the fact that this substance sets in operation the one motor reaction above described. The Paramecium then does not come to rest until the random movements thus caused have carried it by chance, as they must in time, into a region containing none of the substance which causes the motor reaction.

4. Different chemicals have different specific effects upon the various component parts of the above reaction; one part may be intensified or weakened, while other parts are modified in some different way. The motion to or from the chemical is the resultant of these specific effects taken together. The direction of motion, known as positive or negative chemotaxis, is therefore a complex phenomenon, resulting indirectly from various factors.

5. Paramecia are nearest the quiet condition when in a weakly acid solution. Paramecia at rest under normal conditions are in such

a weakly acid solution, due to the carbon dioxide in the water, produced by themselves.

6. When Paramecia in such a weakly acid solution come in contact with a solution containing no acid, the motor reaction (with its reversal of cilia) is produced, so that the Paramecia do not pass out of the acid solution.

7. On passing from a neutral solution to a weakly acid solution no reaction is caused, but if now the Paramecium attempts to pass back again out of the acid solution, the reaction is produced (as in 6), so that the animal remains in the acid solution. The acid thus changes the physiological condition of the Paramecium, so that it now reacts to the neutral fluid.

8. The anions or acid ions present in salts tend to throw the Paramecia into the same physiological condition as the pure acid, so that the Paramecia afterward react when they come in contact with a fluid not containing these ions.

9. It results from 5, 6, 7, and 8 that Paramecia collect in solutions having a weakly acid reaction, or in solutions of salts in which the anion is especially active. Thus the so-called positive chemotaxis of Paramecium is brought about. The positive chemotaxis toward certain nutritive fluids, such as meat extract, is due to the acid reaction of the latter, not to its nutritive value.

10. Alkaline solutions and solutions of salts containing the kations of the alkali metals or of the earth alkali metals produce in a marked degree the motor reactions of Paramecium. As a result the Paramecia do not come to rest until their random movements have brought them into a solution which does not contain a large proportion of these ions. Thus the apparently negative chemotaxis of Paramecia toward these substances is brought about. In a series of salts of any one of the halogens with the different alkali metals, the power of producing the motor reaction (and hence the repellent power) decreases as the atomic weight increases, *except* that potassium salts are throughout more repellent than the lighter sodium salts.

11. In solutions of salts containing the kation of a strong alkali metal, as well as a powerful anion, as of fluorine or chlorine, both of the ions may act on the Paramecia, — the former causing the motor reaction when the Paramecium attempts to enter the solution, the latter when it attempts to leave it. In this case the Paramecia gather in a ring in the outer margin of a drop of the solution and are apparently both repelled and attracted by it.

12. The chief factor causing the motor reaction which results in negative chemotaxis is not the injuriousness of the substance, but is of a chemical nature. Two substances of equally injurious properties have by no means equal repellent powers, but those which are alkaline or contain kations of the alkali or earth alkali elements are much more repellent than most others, without regard to injurious effects.

13. Nevertheless, any substance which directly and severely injures the Paramecia does thereby cause a motor reaction. But this reaction is not produced till the injury has occurred and it is too late to save the Paramecia. They therefore swarm into chemical substances of certain sorts by which they are immediately killed.

14. It is therefore possible to distinguish (1) reactions due purely to the chemical nature of the substance, the reacting Paramecia being entirely unharmed, and (2) reactions due purely to the injury produced by the substance, without regard to its chemical nature. Reactions of the latter class are not sufficiently precise to be protective.

The investigation of which the results are presented in the foregoing paper was pursued in the Laboratory of the United States Fish Commission for the Biological Survey of the Great Lakes, at Put-in-Bay, Ohio, in the summer of 1898, and continued at Dartmouth College during the following autumn. I desire to express my sincere thanks to the officials of the U. S. Fish Commission and to Professor J. E. Reighard, Director of the Survey, for courtesy and assistance in every way during the progress of the work.

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STUDIES ON REACTIONS TO STIMULI IN UNICELLULAR ORGANISMS. V.—ON THE MOVEMENTS AND MOTOR REFLEXES OF THE FLAGELLATA AND CILIATA.

BY HERBERT S. JENNINGS.

STUDIES ON REACTIONS TO STIMULI IN UNICELLULAR ORGANISMS. V. — ON THE MOVEMENTS AND MOTOR REFLEXES OF THE FLAGELLATA AND CILIATA.[1]

By HERBERT S. JENNINGS.

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I. INTRODUCTION.

IN the second of these Studies[2] I have described the mechanism of the motor reactions to external stimuli in the ciliate infusorian Paramecium caudatum. As there set forth, this animal has a fixed form of motor reaction to stimuli of all classes, which, expressed in general terms, is as follows: When unstimulated the animal swims with a certain structurally defined end (the "anterior") in front.

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[1] Scientific Results of a Biological Survey of the Great Lakes, directed by Jacob Reighard, under the auspices of the U. S. Fish Commission, No. V. (Published by permission of the Hon. George M. Bowers, Commissioner of Fisheries.)

[2] JENNINGS: Studies, etc., II. On the Mechanism of the Motor Reactions of Paramecium, this journal, 1899, ii, pp. 311–341.

When stimulated motion takes place with another (the "posterior") structurally defined end in front, followed by turning toward one side which is structurally defined and invariable (the aboral in Paramecium), — finally succeeded by motion with the same end in front as at first. As thus expressed, the direction of motion after a stimulus has no relation to the nature or the position of the stimulating agent.

This method of reaction has a fundamental bearing on the interpretation of the life phenomena of these creatures, and the question is an important one as to how widespread such a style of reaction is among unicellular organisms. In the third of these Studies,[1] an account is given of the reaction methods of the ciliate infusoria Spirostomum ambiguum and Stentor polymorphus, in which it is shown that these animals react in essentially the same manner as Paramecium. Owing to the larger size of these animals, it was possible to localize the action of stimuli very precisely, so that, especially with Spirostomum, even more sharply defined results were obtained than with Paramecium. It was found (1) that Spirostomum reacts to both chemical and mechanical stimuli, *whether applied to the posterior end, the anterior end,* or the side, by swimming *backward* (followed by turning); (2) that the turning is always toward the *aboral* side, whether the stimulus occurs at the anterior end, the posterior end, *the oral side, or the aboral side.* All relation of the direction of motion to the localization of the stimulus seems thus excluded.

I have since made a study of the reactions of a considerable number of other Protozoa, representing various groups of the Flagellata and Ciliata, bringing out thus the more widely distributed reaction methods among these animals, as well as discovering for certain Protozoa modifications in important points of the general reaction method described in the two papers above referred to. The results of this study are given in the present paper.

It will be well to define sharply the main questions to which, in the study of a given Protozoön, answers are desired. These are as follows: —

1. Has the given Protozoön a fixed formula for motor reactions, similar to that of Paramecium?

2. After stimulation, does the organism always turn toward a certain structurally defined *side,* without regard to the nature or position of the stimulus?

3. After stimulation does the organism always move, before turn-

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[1] JENNINGS: American naturalist, 1899, xxxiii, pp. 373-389.

ing, toward a certain structurally defined *end* (the "posterior") without regard to the nature or position of the stimulus?

These questions will serve as directives for the observations and experiments, which will not however be in any way limited by them. I give first a detailed account of the reaction methods of the different Protozoa studied, arranging them according to the groups to which the animals belong. This is followed by an analysis and summary of the observations, showing the variations in the motor reactions among the different Protozoa, and giving as far as possible the answers to the questions proposed above, as well as other general conclusions to be deduced from the facts presented.

II. FLAGELLATA.

The majority of the members of this group are very small and without striking differentiations of structure. It is therefore in most cases very difficult to determine whether the direction of motion after stimulation does or does not have any fixed relation to structural features of the animal's body. A study was made of the reactions of a few species, the results of which are presented herewith.

Chilomonas paramecium Ehr. — *Description.* — Chilomonas paramecium (Fig. 1) is a minute Flagellate which can always be procured in practically unlimited numbers by allowing aquatic vegetation to decay in water. In cultures of this sort Chilomonas is one of the first forms to appear: it is often present in such multitudes as to give the water a milky appearance. The great abundance in which it can be procured at any time make it a most favorable species for experimental studies on the Flagellata. The animal is of an irregularly oblong form, compressed sideways. One end (the "anterior") is usually broader than the other, and bears a shallow notch lying between two "lips." One of these lips (the "dorsal") is much larger and more prominent than the other, so that the anterior end presents the appearance of being obliquely truncate with a notch in the truncate border. From the notch, just beneath the larger lip, arise the two flagella, each of about the same length as the animal's body. The body is sometimes slightly curved, the concavity of the curve being on the so-called dorsal side. All these relations are shown in Fig. 1.

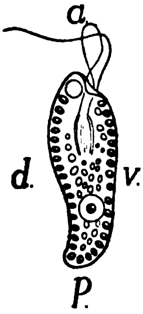

FIGURE 1. Chilomonas paramecium Ehr., right side, after Bütschli. *a*, anterior end; *p*, posterior end; *d*, dorsal side; *v*, ventral side.

Movements. — If a large number of specimens of Chilomonas are mounted and observed under the microscope, they will be seen at first to dart swiftly through the water, — so swiftly that the eye can with difficulty follow them. Some individuals soon come suddenly to rest: others follow, and after a time nearly all the animals are resting against the substratum, beginning movement again only when stimulated. When at rest, Chilomonas is attached by one of its flagella, which is thrown in a coil on the surface of the substratum, while the other may or may not retain a vibratory motion.

More exact observation of the movement brings out the following points. Chilomonas as it swims forward revolves on its long axis in such a way as to describe a path which is a spiral of some width. By introducing the animals into a gelatine solution, so as to compel them to move very slowly, it can be observed that the position of the body bears a constant relation to the axis of the spiral, the lower or smaller lip being always directed toward the outside of the spiral.

Reactions to stimuli. — (1) If to the quiet Chilomonas the faintest possible stimulus be given, as by gently agitating the water, or in any way loosing its hold on the glass, it merely resumes its usual motion, already described. In a certain sense this usual forward motion may then be considered (taking the resting individual as a starting point) as a reaction to the weakest possible stimulus. The motion seems then to be maintained without further stimulus, until the stimulus of contact with a solid (thigmotaxis) again induces the resting condition. The same facts, in accordance with which the usual motion of the animals may be conceived as (at first) a reaction to a weak stimulus, exist in the case of all the organisms studied, and will not be especially mentioned for each. It is usually very difficult to give the resting individual so weak a stimulus as to induce at first only the usual forward motion: as a rule the reaction takes the form to be described immediately.

(2) If now the swimming or resting Chilomonads be stimulated beyond this faintest degree, as by jarring the preparation strongly (mechanical stimulus), by letting the swimming animals come in contact with a diffusing chemical, or dropping them directly into a solution of some chemical (chemical stimulus), reaction takes place as follows. The animals dart swiftly *backward*, then *turn sideways toward the smaller lip*, then swim forward in the new path thus determined by the position of the smaller lip. At the moment of turning toward the smaller lip, the flagella, or at least one of them,

are turned toward the dorsal side, over the upper lip, as indicated in Fig. 2.

The point of essential importance here is of course the fact that the animals always turn when stimulated toward the smaller lip, without regard to the nature of the source of stimulus or its position. This is easily seen when the Chilomonads are examined in the thickened gelatine solution, in which all their motions are slow; it is much less easily made out in the animals swimming freely in water, though in all cases observed in which it was possible to determine under these conditions in which direction the animal turned, it was evident that this was toward the lower lip.

The method of reaction is very easily observed under the following conditions. A drop of water containing the animals is spread out in a thin layer on a slide and is left uncovered. The animals in this thin layer of water may then be watched with the high power of the microscope. As the water evaporates, the partial desiccation of the individuals near the edge of the drop acts as a stimulus; they dart backward, turn toward the smaller lip, swim forward, and repeat the operation indefinitely. At this time the layer of water has become so thin that the animals can swim in only one plane and are forced to lie upon one side; it is therefore at once apparent in which direction they turn, — in every case toward the smaller lip.

As in the case of Paramecium, the different parts of the reaction may be modified in intensity and duration by different conditions. Chilomonas usually swims backward only a short distance, but the distance thus travelled varies somewhat with the intensity of the stimulus, being greater with a powerful stimulus. As the animal swims backward it usually does not revolve at the same time on its long axis, though in the more extensive backward excursions it may do so. The amount through which the animal *turns* is very variable; with a faint stimulus there is a turn of but a few degrees toward the lower lip, while with a powerful stimulus, as when the animals are dropped directly into a strong chemical, or partially desiccated in the manner described, they may whirl about so as to describe a circle several times. When dropped into a weak solution of sodium chloride the entire reaction is repeated many times, — until finally the turning prevails over the other features, and the animals whirl about until they die.

When a Chilomonas swimming forward comes in contact with the edge of a drop of some diffusing chemical, as $\frac{1}{5}$ per cent sodium chloride, the reaction is given as above described, — the Chilomonas after reaction of course continuing to swim forward in a new direction. If this new direction, as frequently happens, again brings the animal against the edge of the drop, the reaction is repeated, and this repetition is continued until in accordance with the laws of chance, the animal's movement becomes so directed as not to carry it against the edge of the drop. These relations are identical with those described in the second of these Studies for Paramecium.

If a crystal of sodium chloride is placed against the edge of a cover-glass beneath which are large numbers of Chilomonads, as the diffusing salt passes under the cover-glass the following effects are observed. The Chilomonads affected all swim backward at once. Those whose anterior ends are directed away from the advancing salt solution, swimming backward, pass directly into the solution, and are killed. Those with anterior ends directed toward the crystal of sodium chloride, likewise swimming backward, move away from the diffusing chemical and therefore escape. Those with the body axis in intermediate directions, swimming backward, move obliquely with reference to the position of the advancing salt solution; some thus escape, while others are killed. The direction of motion bears no relation to the position of the source of stimulus, — the stimulated animals all swimming (at first) simply backward.

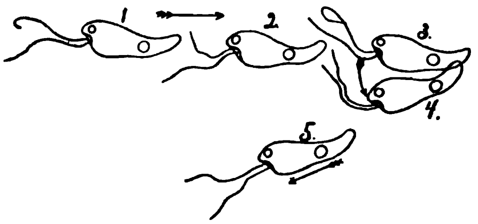

FIGURE 2. Motor reflex of Chilomonas. The arrows show the direction of movement, while the numbers show the successive positions taken.

The typical motor reflex in this animal may then be expressed as follows: Chilomonas when stimulated swims backward, turns toward its lower lip, then swims forward in the direction thus determined. The typical motor reflex of Chilomonas is shown in Fig. 2.

A comparison of the facts above given as to the movements of Chilomonas with those detailed in the second of these Studies for Paramecium reveals a complete agreement in all essential points between the motor reactions of the two, — the one a Flagellate, the other a Ciliate.

Euglena viridis Ehr. — *Description.* — The well-known form Euglena viridis (Fig. 3) is an elongated, somewhat fusiform creature, exceedingly flexible, and variable in shape. One end (the "posterior") is pointed; the other (the "anterior") is somewhat obliquely truncate, there being, as in Chilomonas, a larger and a smaller lip, with a notch between them. From this notch or mouth projects a single long flagellum. Just behind the larger lip and a little to the left of it lies a red eye-spot.

Movements. — When in undisturbed motion Euglena swims forward, revolving on its long axis and describing a slender spiral.

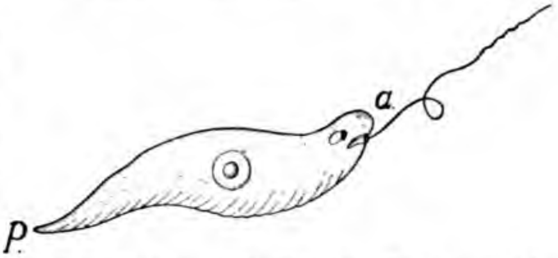

FIGURE 3. Euglena viridis Ehr., after Kent. *a*, anterior end; *p*, posterior end.

Reactions to stimuli. — When Euglena thus swimming is given a weak stimulus, as by its coming in contact with a drop of some weak chemical, or by a mechanical jar to the preparation, *it turns toward the larger lip*, then keeps on the course so laid out undisturbed. Euglena never swims backward under any circumstances, so far as observed. If the stimulus is a little stronger, the creature may stop for a moment before turning. If the stimulus is still stronger, the Euglena continues turning, so that it frequently revolves thus for a long time, toward the larger lip, without any forward motion at all. If the stimulus continues, the whirling Euglena gradually takes a spherical form, the motion ceases, and the creature proceeds to encyst itself. To any very strong stimulus the only response is the immediate assumption of a spherical form and prompt preparation for encystment. This occurs when the creatures are introduced into a chemical solution, as of NaCl, KI, etc., or when allowed to dry on the slide.

The motor reaction of Euglena consists then in simply turning toward the larger lip. Further reaction takes the form of preparation for encystment.

In this case then, as in those previously described, the direction of motion after a stimulus is determined by structural differentiations of the body, not by the position of the stimulating agent.

Other Euglenidæ. — Several other species of Euglena are common in fresh water; of these Euglena spirogyra and Euglena oxyuris were studied. These creatures are much more sluggish than Euglena viridis, moving only very slowly and lying quiet on the substratum for long periods; they seem much more vegetable than animal in their nature. In these creatures no definite motor reaction to a stimulus could be observed. When stimulated they either do not react at all, or simply assume the spherical form and make preparations for encystment.

In several species of Phacus and certain other green Flagellates no definite motor reaction plan could be made out; these organisms are worthy of further study in this connection.

In these Flagellata we have therefore a gradation in the complexity of the motor reaction to a stimulus. In Chilomonas we find all the features exhibited by Paramecium, — the swimming backward, turning toward one side, and again swimming forward; continued or severe stimulus causing repetitions of the reaction. In Euglena viridis the swimming backward is omitted, the creature merely turning toward one side when stimulated; continued or severe stimulus induces encystment. In Euglena spirogyra and E. oxyuris even the turning is omitted, there being little or no response to the stimulus but immediate preparation for encystment.

III. CILIATA.

A. Holotricha: Paramecium caudatum Ehr. — An extended account of the motor reactions of this animal has been given in the first, second, and fourth[1] of these Studies.[2] For our present purpose the following statement of previous results is sufficient: Paramecium when stimulated swims backward, turns toward its own aboral side, and swims forward.

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[1] JENNINGS: This journal, 1899, ii, pp 355-379.

[2] The results given in section 3 of the fourth of my Studies (this Journal, 1899, ii, pp. 363-371) on the Relation of Chemotaxis to Chemical Composition in Paramecium require modification, owing to a chemical misinterpretation as to the factors which give solutions their characteristic properties. My discussion was based on the old ideas that the acid properties of solutions are due to the "acid radicals," the alkaline properties to the metal components. But modern chemistry has shown that the characteristic properties of acids are due to the hydrogen ions contained; of alkalies to the hydroxyl ions. This requires changes in my discussion and conclusions, and renders it probable that little or no significance is to be attached to the results as to the attractiveness of certain salts given in Table II, page 366 (*loc. cit.*); since, as was stated in that paper on page 369, they are inconstant. The only solutions classified as always attractive in my list on page 362 that are known not to contain hydrogen ions when pure are those of sodium and potassium fluoride, and of potassium permanganate. Tests of the samples of these substances used in my experiments have since shown that these, though

It is, perhaps, well to call attention again at this point to the fact that, taking the resting individual as the starting point, the usual straightforward motion may be considered as the simplest form of reaction to a very weak stimulus. Such a stimulus may most easily be given in the case of Paramecium by gently removing with a fine glass spicule the bit of material against which the animal is resting, or by a gentle rolling movement separating the Paramecium from this bit of material. Thereupon the animal resumes its forward course. This forward motion, however, continues long after the cessation of the external change which induced it, so that it may be proper to speak of the animal swimming forward in the normal manner as " unstimulated."

In addition to details previously given concerning the reactions of Paramecium, I wish to add here certain new results, which are of importance for interpreting the phenomena presented by the reactions of this and other unicellular organisms.

Localized stimuli. — (*a*) *Mechanical.* — With the new Braus-Drüner stereoscopic microscope of Zeiss, it is possible to apply localized stimuli to Paramecium, in the manner described in the third of these Studies (*loc. cit.*) for Spirostomum and Stentor. Under the Braus-Drüner microscope the Paramecia in a watch-glass are touched on various parts of the body with the tip of the finest capillary glass rod. By this means it is found that only the anterior end of the

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procured as strictly chemically pure from a reliable firm, were contaminated with acid; after proper purification they were repellent throughout. All the solutions in which the Paramecia gather, therefore, contain hydrogen ions, and it is doubtless to these that the (apparently) attractive qualities are due, instead of to the anions as stated in the paper. On the other hand, my conclusion that the strong repellent power of other solutions is due to the metal components or kations rather than to the OH-ions seems justified, since of the thirty-six substances classified as having strong repellent powers, all contain such kations, while at most less than a dozen contain OH-ions. It is, however, not impossible that OH-ions are repellent also.

In my discussion and conclusions, therefore, wherever an effect was ascribed to the anions or acid radicals, it should be considered due rather to the hydrogen ions. Of the fourteen numbered paragraphs in which I have summed up my conclusions in this paper (*loc. cit.*, pp. 377–379), three — paragraphs 8, 9, and 11 — are to be modified in accordance with these facts. The other conclusions are not modified.

animal is markedly sensitive. When touched at the anterior end the Paramecium quickly gives the typical reaction previously described. But if any other part of the body is touched, the Paramecium does not react at all: it may be pushed out of its course, but does not of itself change its path in the least, in consequence of the touch.

It must be remembered, that owing to the minute size of Paramecium, it is impossible to give it a blow of any force; the creature is simply pushed aside by any object touching it, — just as a bubble of this size cannot be broken by touching it with a rod, in spite of its extreme delicacy. It is quite impossible to cut, bruise, or otherwise injure Paramecium in this way, so that the lack of reaction is doubtless due to the fact that the touch is too light to be perceived, except when it occurs at the sensitive anterior end.

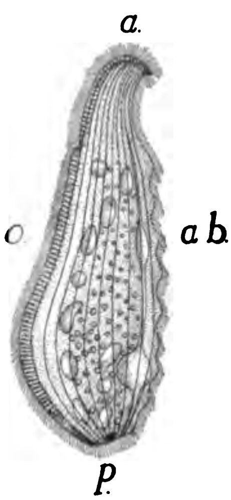

FIGURE 4. Loxophyllum meleagris O. F. M., after Bütschli. *a*, anterior end; *p*, posterior end; *o*, oral side; *a b*, aboral side.

(*b*) *Localized chemical stimuli.* — For determining the effects of localized chemical stimuli, a capillary glass rod was coated with the chemical to be tested and held near the animals, at one end or one side. The chemical then diffuses, and the effect when it reaches the animal can be noted. It is convenient to use as a stimulus some colored chemical, so that its diffusion can be seen; methyl green serves well for this purpose.

Holding the glass rod thus coated with methyl green *behind* a number of Paramecia, all of which are resting with their anterior ends placed against a solid (thigmotaxis), it is found that as soon as the diffusing chemical reaches the animals, coming of course first in contact with their posterior ends, they at once *swim backward,* — therefore into the densest part of the solution, where they are killed.

If the methyl green is held in front of them, so that it first comes in contact with the anterior end, the Paramecia likewise swim backward, — therefore *away* from the densest part of the solution, so that they escape.

If a bit of the methyl green or a crystal of sodium chloride is placed in the midst of a group of Paramecia that are oriented in no particular direction, each individual begins to swim backward when the diffusing chemical reaches it. Some thus swim toward the centre of diffusion of the chemical, others away from it, others obliquely, — exactly as described for Chilomonas.

It does not follow, however, from the above experiments that when Paramecium swims backward into a chemical solution which first touches its posterior end, this reaction is due to a stimulus at the posterior end. It is possible that when the diffusing chemical approaches the Paramecium from the rear, no reaction is caused *till the solution reaches the anterior* end. It is impossible to determine by observation whether this is true or not, even with a colored substance like methyl green, as the Paramecia usually begin to react before the color can be seen to have reached them at all. The possibility remains then that in Paramecia all motor reactions may be due to a stimulus at the anterior end, — the rest of the body not being sufficiently sensitive to receive a stimulus at all.

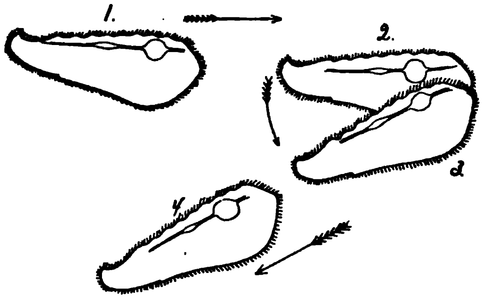

FIGURE 5. Motor reflex of Loxophyllum meleagris. The arrows give the direction of motion; the numbers show the successive positions occupied.

Loxophyllum meleagris O. F. M. — *Description.* — Loxophyllum meleagris (Fig. 4) is of a flattened leaf-like form, about three times as long as broad, the anterior end tapering to a point, the posterior end broad. One border (the oral or ventral) is extremely thin; the other (aboral or dorsal) thicker and bearing a series of papillæ. The anterior end usually curves toward the aboral side.

Movements. — This animal usually glides along the substratum, lying on one side, — after the manner of the Hypotricha. Like the Hypotricha, it may also at times leave the bottom and swim freely through the water: at such times it revolves on its long axis from left to right. The gliding motion is the usual one, however.

This gliding motion is either straight ahead or in a gentle curve toward the aboral side, — the latter being evidently due to the curvature of the anterior part of the body. The straight forward movement seems to occur in individuals in which the curving of the anterior end is not marked.

Motor reactions. — When Loxophyllum comes in contact with an obstacle of any sort, it turns toward the thin *oral side.* If stimulated in other ways, as by a mechanical shock, or by a chemical stimulus, it may swim backward a short distance, then turn toward the oral side. The reaction of Loxophyllum is represented in Fig. 5.

It will be noticed that in this case the path of the unstimulated animal takes (frequently) the form of a curve toward the aboral side, while a stimulus causes always a sudden turn toward the opposite, or oral side.

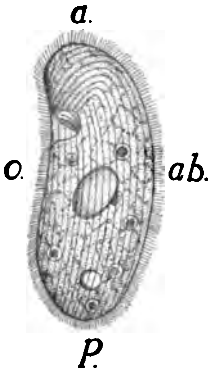

FIGURE 6. Colpidium colpoda Ehr., after Schewiakoff, from Bütschli. *a*, anterior end; *p*, posterior end; *o*, oral side; *a b*, aboral side.

Colpidium colpoda Ehr. — *Description.* — Colpidium colpoda (Fig. 6) is somewhat kidney shaped, narrower at the anterior end, two or three times as long as broad, and curved toward the side on which the mouth lies (the oral side).

Movements. — In Colpidium we find the same phenomenon in the direction of the normal movement as in Loxophyllum, but more pronounced. The animal swims in a strong curve forming the arc of a circle, with the oral side toward the centre of the circle. This is most evident when the animal is following a plane surface, keeping one side against the surface, as it frequently does. When swimming freely through the water it likewise swerves continually toward the oral side, at the same time revolving slowly on its long axis, so that its path takes the form of a wide spiral. The curving of the path toward the oral side seems clearly a consequence of the curved form of the body.

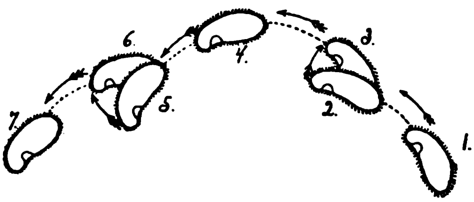

FIGURE 7. Path of Colpidium when frequently stimulated. The arrows show the direction of motion; the numbers give the successive positions taken.

Motor reactions. — When stimulated in any way, Colpidium turns toward its aboral side. This may be observed in the usual course of the animal as it glides along a surface. As above stated, its undisturbed path is a curve toward the oral side, but as it at short intervals comes in contact with an obstacle or is otherwise stimulated, it gives a short jerk toward the aboral side. Its path therefore usually takes the form shown in Fig. 7.

The reaction is made more evident by using some direct means of stimulating the animals. If a drop of some chemical, as a weak solution of NaCl, is introduced beneath the cover-glass of a preparation of Colpidia, the latter always turn toward the aboral side when they come against the edge of the drop: this is true whether the animals are gliding along a surface or swimming freely through the water. The backward swimming, so noticeable as a part of the reaction in many forms, is nearly omitted here; if the stimulus is strong there is a sudden stoppage before the turning, with at times a slight backward jerk.

If the animals are dropped directly into a solution of a chemical which acts as a stimulus, they begin at once to whirl about, turning continuously toward the aboral side.

Putting all together, the movements and reactions of Colpidium are then as follows: the unstimulated animal swims in a curve which follows the curve of the animal's body, turning therefore continually toward the oral side; at any effective stimulus the animal alters its course by turning toward the aboral side (at times jerking a little backward at the same instant).

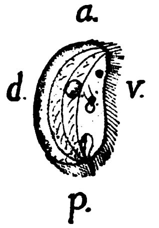

FIGURE 8. Microthorax sulcatus Eng., after Engelmann. *d*, dorsal edge; *v*, ventral edge; *a*, anterior end; *p*, posterior end.

Microthorax sulcatus Eng. — *Description.* — Microthorax (Fig. 8) is very small, and somewhat lens shaped, one surface flattened, the other convex and bearing three longitudinal grooves. The anterior end has a blunt point curved toward the ventral edge, the posterior end is rounded. The "dorsal" edge is strongly convex, the ventral edge nearly straight.

Movements. — Microthorax usually creeps or swims on the substratum, lying either on the flat surface or the convex surface; perhaps rather more commonly on the flat surface. The path is not a straight one, but forms a curve, the animal continually turning toward its more convex edge (dorsal). When swimming freely through the water Microthorax revolves continually on its long axis; at the same time it swerves toward the convex edge, so that the path becomes a spiral one.

Motor reactions. — When Microthorax meets an obstruction or comes in contact with water containing some effective chemical in solution, or is otherwise stimulated, it turns with a sudden jerk *toward the convex (dorsal) edge,* then goes forward again. The sudden turn may or may not be accompanied by a backward jerk. Microthorax never turns toward its straight (ventral) edge.

Dileptus anser O. F. M. — *Description.* — Dileptus anser (Fig. 9) is lanceolate in form, pointed at the posterior end, and extending at the anterior end into a long, somewhat trunk-like portion. On one side (the oral) lies the mouth; along the opposite side (the aboral) is a row of contractile vacuoles. The anterior trunk-like portion is slightly curved in such a way that the convexity of the curve lies on the oral side, — the tip being thus directed toward the aboral side.

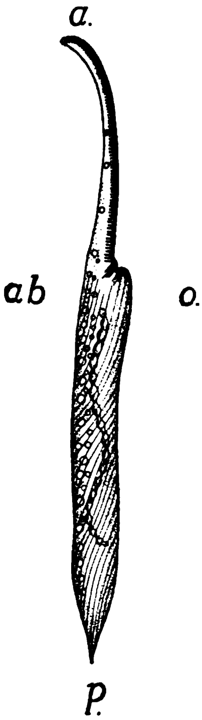

FIGURE 9. Dileptus anser O. F. M., after Wrzesniowski, from Bütschli. *a*, anterior end; *p*, posterior end; *o*, oral side; *a b*, aboral side.

Movements. — When swimming freely through the water the animal revolves on its long axis in such a way that it describes nearly the surface of a cone, the posterior end forming the apex, while the convex curve of the anterior process (oral side) looks always toward the outside of the cone. In other words, the anterior end swerves continually toward the oral side, the continued revolution carrying the animal forward in a spiral course.

Dileptus may also contract and extend and twist itself about in very irregular ways, though so far as observed the oral side may always be seen to look toward the outside of the irregular curves formed.

Motor reactions. — When Dileptus in its forward course strikes against an obstruction or comes in contact with the edge of a drop of some chemical solution, it swims backward, then turns toward the oral side, — toward the convex curve of the proboscis-like anterior region, — then swims forward over the new path so determined.

Rarely the animal becomes so twisted that the convex curve or oral side of the proboscis comes to lie nearly in a line with the aboral side of the remainder of the body. In such a case it is toward the oral side of the proboscis that the animal turns, without regard to the posterior part of the body.

Localized stimuli. — (*a*) *Mechanical stimuli.* — Dileptus is of such a size that the effects of localized stimuli may be tried, in the manner above described for Paramecium. Touching the anterior end with the spicule of glass, it is found to be exceedingly sensitive, the reaction described above being given at the slightest touch. If the posterior part of the body is touched, usually no reaction is given, showing that, as in Paramecium, this part of the body is very little sensitive as compared with the anterior end. By a strong stimulus, however, the animals may be made to react by touching the posterior end, but *they then swim forward*, instead of giving the typical reflex above described. While it seems to be only a small part of the anterior end that is especially sensitive, a sufficiently strong stimulus on any part of the anterior half of the body causes the animal to swim backward, while a strong stimulus applied to any point back of the middle of the body causes Dileptus to swim forward.

Thus in Dileptus the reaction to a mechanical stimulus varies with the position of the stimulus, — a stimulus in front causing backward motion, a stimulus behind causing forward motion. These results thus differ from those previously obtained for Spirostomum (see Studies, etc., III, *loc. cit.*); in Spirostomum an effective stimulus at any point of the body caused a contraction followed by swimming *backward.*

In Dileptus we have therefore a reaction with relation to the localization of the stimulus. But strangely enough this reaction with reference to the localization of the stimulus occurs, as will be shown, (1) only in relation to backward and forward motion, not in relation to movement sideways; (2) it occurs also only in the case of mechanical stimuli, there being no sign of it in the case of chemical stimuli.

(1) As to the first point, we have now to consider the results when the stimulus is applied on one side. If the animal is stimulated on the anterior half of the body, it swims backward, turns to the oral side, and swims forward. If the stimulus is a light one, the backward motion is for only a fraction of the animal's length, when turning takes place. Now, if the animal is stimulated on the oral side, it persistently turns toward that side. The stimulus may be repeated, the animal receiving a series of slight taps; it then turns continually *toward* the rod which is giving the stimuli. If on the other hand a series of stimuli is given in the same way on the aboral side, the animal continually turns *away* from the stimulating agent. There is not the slightest indication of reaction with reference to the localization of the stimulus so far as the direction of turning is concerned. Dileptus when stimulated always turns toward the oral side if it turns at all.

(2) As to the second point, we may proceed to an account of the effects of

(*b*) *Localized chemical stimuli.* — If a crystal of NaCl or a capillary glass rod coated with methyl green is held in front of a resting Dileptus, there is for a moment no response. Then as the diffusing chemical reaches the animal, it darts backward, thus escaping from the advancing flood without injury.

If the chemical is placed some distance behind the Dileptus, as the diffusing chemical reaches it the animal *darts backward*, as before, thus approaching and entering the dense solution, where it may be killed. If the chemical solution comes from one side, the same reaction is given, — the direction of motion being then neither toward nor away from that from which the solution is coming. If the animals are dropped directly into a weak chemical solution, the reaction is likewise the same.

There is thus no sign in the reaction to a chemical of any relation to the localization of the stimulus. It seems probable that no reaction occurs until the diffusing chemical has reached the sensitive anterior end of the animal; then the typical reflex is given without relation to the direction from which the chemical came.

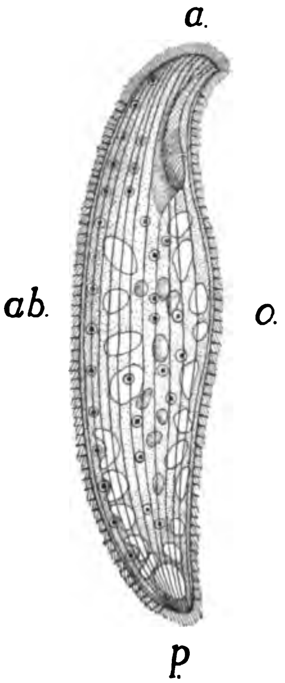

FIGURE 10. Loxodes rostrum O. F. M., after Bütschli. *a*, anterior end; *p*, posterior end; *o*, oral side; *a b*, aboral side.

Loxodes rostrum O. F. M.—*Description.*— Loxodes rostrum (Fig. 10) is a very large infusorian, of a flattened elongated form, the anterior end pointed and curved toward the oral side; the posterior end likewise pointed or sometimes blunt. One of the sides is convex, the other deeply furrowed. The mouth lies on one edge (the oral), not far from the anterior end. Owing to its large size and the slowness of its motion, Loxodes is an especially favorable form for observing the effects of localized stimuli.

Movements. — Loxodes moves very slowly, at times creeping along the substratum on one side, at times swimming freely through the water. In the former case the path is nearly or quite straight; in the latter case the animal revolves and the path is a spiral, the aboral edge lying to the outside of the spiral.

Motor reactions. — When Loxodes strikes with its anterior end against an obstruction, or thrusts it suddenly into a solution of some chemical, it swims slowly backward and turns to the aboral side.

Localized stimuli. — (*a*) *Chemical.* — If a crystal of sodium chloride is dropped near the anterior end of Loxodes, it swims backward (thus away), turns to the aboral side, and swims forward. If the salt is dropped near the posterior end, the Loxodes, when the diffusing solution reaches it, swims backward, as before. It thus approaches and enters the densest part of the solution, where it may be plasmolyzed and killed.

(*b*) *Mechanical stimuli.* — Touched on the anterior part of the body with the glass rod, Loxodes gives the complete typical reaction. Touched on the posterior part, it swims forward. Touched on the aboral side, the animal turns persistently *toward* the stimulating agent; on the oral side, it turns *away* from the stimulating agent. Thus it always turns toward the aboral side.

The facts as to the reactions of Loxodes are thus precisely parallel to those of Dileptus. The animal reacts with reference to the localization of mechanical stimuli so far as backward and forward motion is concerned, but not so far as movement sideways is concerned. In the case of chemical stimuli there is no relation to the localization of the stimulus.

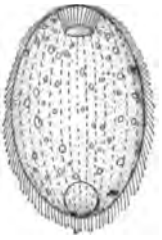

FIGURE 11. Prorodon niveus Ehr., modified from Ehrenberg.

Prorodon. — In all of the Protozoa thus far discussed, there is a more or less striking asymmetry in the form of the body, and this asymmetry is directly correlated with the method of reaction, so that the direction in which the animal turns after receiving a stimulus may be expressed in terms of the animal's structure. But there are a number of infusoria in which no such asymmetry is to be observed, the animals presenting apparently a complete radial symmetry. Such is the case for example with Trachelomonas among the Flagellata: such also is nearly or quite the case with Coleps hirtus and with the various species of Prorodon (Fig. 11) among the Ciliata. The reactions of a number of such forms were studied with care, but in most cases it was impossible to tell whether the animal does or does not turn after stimulation always toward the same side, — the different sides not being distinguishable.

Finally a large species of Prorodon was secured which presented the elements necessary for a solution of the problem.

Description. — Prorodon (outlines of a certain species of which are shown in Fig. 11) is oval in form, with the pharyngeal tube at the anterior end, so that it is not possible to structurally distinguish different sides. It is therefore of course impossible to tell, from the structure alone, whether the animal after stimulation always turns toward a definite side or not. But here the food vacuoles come to the rescue. The animals contained numerous food vacuoles of different size and color, and these occupied definite positions within the body. The movement of the food vacuoles was so slow as not to be apparent except upon long observation. By fixing upon one of these vacuoles, occupying a definite position near one side of the animal's body, it was possible to determine in every case the direction of movement of the animal with reference to the position of this vacuole. Upon doing this, it is found that Prorodon, like the other infusoria, always turns toward the same side.

Movements. — The motion of the unstimulated Prorodon is straight forward, at the same time revolving on the long axis to the left.

Motor reactions. — When stimulated it first swims backward, — sometimes a very little, sometimes a long distance. Then it turns. One specimen whose movements were studied contained near one side a large brown food vacuole, — the other food vacuoles being much smaller and less deeply colored. In every case this animal turned after stimulation toward a point in its body surface lying about fifty degrees to the right of the position of this large brown vacuole. In other words, if a plane is passed through this vacuole and the longitudinal axis of the Prorodon, and another plane cutting the first at an angle of about fifty degrees is likewise passed through the long axis, the animal always turned after stimulation in this second plane. Moreover, it always turns in the same direction in this plane, — in a direction definable as follows: when the brown food vacuole lies in the *upper* surface the direction of turning is somewhere in the right half of the animal; if the vacuole is below, the turning is to the left. Similar results were obtained by a study of other individuals.

It thus appears that the radially symmetrical Prorodon likewise always turns toward a definite side. Though anatomically symmetrical, it is physiologically unsymmetrical.

B. Heterotricha: Stentor polymorphus Müller. — An account of the reactions of this animal was given in the third of these Studies (*loc. cit.*). The reaction to a stimulus is essentially as follows: the animal contracts, swims backward, turns to the right, and swims forward.

Spirostomum ambiguum Ehr. — The reactions of Spirostomum were likewise described in the third of these Studies. When stimulated, the animal contracts, swims backward, turns toward the aboral side, then swims forward.

In the case of localized stimuli, the Spirostoma previously described (*loc. cit.*) reacted in the same manner whatever the part of the body stimulated. Stimuli at the anterior end, at the posterior end, or on one side, caused the characteristic reflex, including the swimming backward. In view of the different results obtained for Dileptus anser, Loxodes rostrum, and the Hypotricha, as detailed in this paper, I have been at some pains to re-examine the reactions of Spirostomum. I can confirm the results previously given, from experiments on specimens from several cultures. As noted in the previous account, the posterior end is slightly less sensitive than the anterior end, and the percentage of cases giving the typical reaction was slightly less in the case of stimuli at the posterior end, as compared with the results of stimulation at the anterior end. In specimens from some cultures the difference in reaction to stimuli at the two ends was greater, but in the majority of cases a mechanical stimulus at the posterior end caused the animals to contract and swim backward, exactly as does the same stimulus at the anterior end or the side.

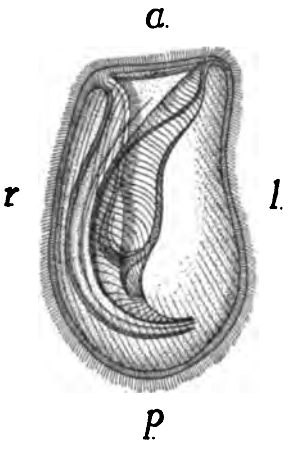

FIGURE 12. Bursaria truncatella O. F. M., after Schuberg, from Bütschli, ventral view. *a*, anterior end; *p*, posterior end; *r*, right side; *l*, left side.

To what is this difference between Spirostomum on the one hand and Dileptus, Loxodes, and the Hypotricha on the other, in regard to reactions to localized mechanical stimuli due? Spirostomum is a very long slender form, and the posterior part seems relatively much more sensitive than in the other cases; it seems probable that to this greater sensitiveness of the posterior end is due the production of the typical reflex when the posterior part is stimulated. Spirostomum differs from most of the others in that it always contracts strongly before responding with the motor reaction; possibly this contraction and the typical motor reflex with its backward swimming are closely bound up together, so that whatever causes the former must cause the latter also.

Bursaria truncatella O. F. M. — This very large Ciliate has proved much less favorable for experimental work than was expected, owing to its extreme delicacy. To the slightest unfavorable influences or mechanical injuries it succumbs quickly, passing first into a pathological condition, then rapidly going to pieces.

Description. — Bursaria (Fig. 12) is ovate, truncate anteriorly, broader and rounded posteriorly. The oral side is flattened, with a very deep groove passing far into the substance of the body; the aboral side is strongly convex.

Movements. — The animal usually swims forward, revolving to the left. At times it jerks back a little (probably as a response to a slight stimulus); at the same time the revolution may partly or completely stop. At times it swims forward for some time without revolving; at such times the path is in some individuals a straight line, in others a regular curve. The direction of the curve probably depends (as in Colpidium and Loxophyllum) on the form of the animal's body. In almost all cases observed the path was a gentle curve to the right (the oral side being considered as ventral). In one or two cases of individuals which had been kept for a long time and were possibly in a pathological condition, the forward path was a curve to the left.

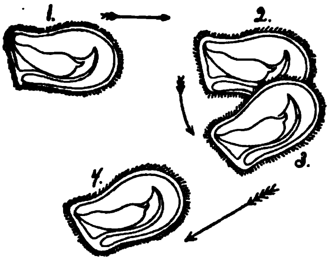

FIGURE 13. Motor reaction of Bursaria truncatella. The arrows show the direction of motion throughout the reaction, while the numbers indicate the successive positions occupied.

Motor reactions. — Bursaria is excessively sensitive to external influences: at any stimulus it swims backward more or less, then turns to the right, and swims forward. The turning to the *right* as a response to a stimulus is invariable whether the regular course is a straight line, a curve to the right, or a curve to the left. Bursaria confined under a supported cover-glass can detect the difference in the water as it nears the edge of the cover-glass nearly a millimetre from the edge. It then turns, always to the right, — even though its right side already lies next to the edge of the water, so that it is compelled to turn in this direction more than 180° to avoid the edge, while to the left it would have had to turn through but a small angle.

The reaction of Bursaria is represented in Fig. 13.

C. Hypotricha: Oxytricha fallax Stein. — *Description.* — Like most of the Hypotricha, Oxytricha (Fig. 14) has a strongly dorso-ventrally flattened body, the dorsal surface being convex, the ventral surface plane. The cilia and cirri serving for locomotion are confined to the ventral surface. The mouth is situated on the ventral surface a very little in front of the centre; from it a row of large cilia, the adoral zone, passes forward and to the left as far as the left corner of the anterior margin; thence it passes along this margin to the right corner. The posterior portion of the animal is distinctly broader than the anterior portion. Anterior and posterior ends, right and left sides, and dorsal and ventral surfaces are thus strongly marked and easily distinguishable.

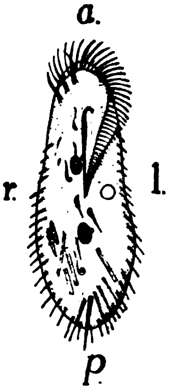

FIGURE 14. Oxytricha fallax Stein, after Kent, ventral view. *a*, anterior end; *p*, posterior end; *r*, right side; *l*, left side.

Movements. — Oxytricha usually runs along the substratum with ventral side applied to it, by means of the ventral cirri. When running along a plane surface, the path followed is in some individuals a straight line, in others a curve to the right, in others a curve to the left. Whether the path is a straight line or a curve, and in the latter case the direction of curvature probably depends partly or entirely upon the form of the given individual; some specimens in which the body was curved distinctly to the left followed a path that was likewise curved to the left. For a given individual traversing a plane surface the direction of the curvature was constant, so far as observed, although the degree of curvature of the path varies in one and the same animal.

Individuals running about on an irregular surface amid detritus, algæ, etc., may follow the substratum in any direction it takes them, running around the surface of spherical or irregular masses, or following a filamentous alga as it curls to the right or left, or up and down. The direction of movement seems determined by the form of the substratum on which the animals are moving.

Besides this usual motion along the substratum, Oxytricha may swim freely through the water, at the same time revolving on its long axis.

Motor reactions. — (*a*) *Mechanical stimuli.* — As Oxytricha runs along the substratum it frequently comes in contact with small obstructions; it thereupon jerks slightly backward and *turns to the right* (that is, toward its own right side), then again pursues a forward course. Similarly if some moving infusorian strikes in its course against the Oxytricha, the latter jerks back and turns to the right. If the preparation containing the moving animals is jarred, they all jerk backward, turn to the right, and move forward. As Oxytricha moves along the substratum it will frequently be seen thus to jerk back and turn a little to the right, there being probably invisible sources of stimuli affecting the animal. The turning to the right after such a jerk backward is invariable, whether the original path was a straight line, a curve to the right, or a curve to the left. In long continued observation of many individuals I have never yet seen one turn otherwise than to the right after a stimulus. The typical motor reflex of Oxytricha is shown in Fig. 15.

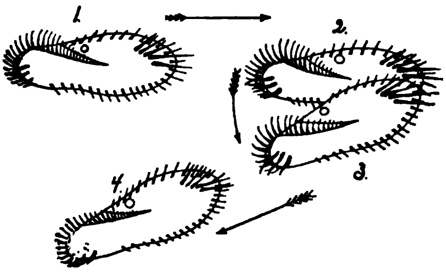

FIGURE 15. Motor reflex of Oxytricha fallax, viewing the animal from the ventral side. The arrows give the direction of motion; the numbers show the successive positions occupied.

(*b*) *Chemical stimuli.* — If a weak solution of some chemical, as sodium chloride, is allowed to run beneath the edge of the coverglass, so that there is a sharply defined boundary between the edge of the drop and the water containing the infusoria, many of the latter will in their course come against the edge of the drop. They thereupon turn to the right. This turning to the right occurs both when the animals strike with the anterior end squarely against the edge of the drop, and when they come against it obliquely. Cases were observed in which the Oxytricha simply grazed the edge of the drop with its right side; it nevertheless after jerking backward turned to the right. In such a case, if the forward course again leads the animal against the edge, it turns to the right again. It is evident that continued turning to the right will soon bring the animal completely around, so that it will be headed in the opposite direction.

Animals swimming freely through the water and at the same time revolving on the long axis, likewise turn to the right when they come in contact with the edge of a drop of some chemical in solution. If they are dropped directly into a solution of sodium chloride they jerk backward, turn to the right, and so continue for a long time, — the whole effect being that of a jerky whirling toward the right. Dropped into 6 per cent solution of sodium hypochlorite, they all whirled steadily and smoothly to the right. Some other chemicals cause the animals to swim backward a considerable distance before turning to the right; doubtless variations in the effects of chemicals similar to those described in the fourth of these Studies (*loc. cit.*) for Paramecium might be worked out for Oxytricha.

Localized stimuli. — (*a*) *Mechanical.* — Touching various points on the surface of the body with the capillary glass rod, the anterior end is found to be much more sensitive than the rest of the body. The slightest touch at the anterior end — apparently even a touch to one of the cirri of this region — causes a strong reaction. The posterior part of the body is much less sensitive; the tip of the glass rod may even be thrust so violently against it as to leave a visible mark, without any strong reaction being caused. The animal reacts indeed, but quietly as compared with the violent movement when the anterior end is touched.

Touched at the sensitive anterior end Oxytricha reacts by darting backward, turning to the right, and swimming forward again (typical motor reflex).

Touched on the posterior half of the body, usually no reaction is caused. If a strong blow is given with the tip of the capillary glass rod sufficient to make a visible mark, the animal responds by *running forward.*

Touched on the left side, the animal jerks back a little, then turns to the right (away from the stimulating agent).

Touched on the right side, Oxytricha jerks back a little and turns to the right, as before, — therefore toward the stimulating agent. The rod may give repeated quick taps on the right side; the animal turns persistently to the right, as if following the rod. If touched repeatedly on the left side, it of course turns as persistently away from the rod.

Thus Oxytricha, like Dileptus and Loxodes, reacts with reference to the localization of the stimulus so far as backward and forward motion is concerned, but not so far as motion sideways is concerned: it turns toward a structurally defined side, without regard to the place of stimulation.

(*b*) *Localized chemical stimuli.* — As already shown, Oxytricha responds to a chemical stimulus which first affects the anterior end or the side by backing a little, turning to the right, then swimming forward, — without regard to which side it is that first comes in contact with the chemical. There remains to be described the reaction to a chemical stimulus coming from behind. If a crystal of sodium chloride or a rod coated with methyl green is placed behind the resting Oxytricha, there is for a moment no response; then as the diffusing chemical reaches the animal, the latter at once swims backward. It thus passes into the densest part of the solution and may be killed. Usually after swimming some distance backward, this motion is exchanged for a whirling motion to the right. If the chemical is placed in front of Oxytricha, it darts backward, as before, — thus away from the diffusing chemical. If the animals are introduced directly into the chemical, they fall at once to swimming backward, which usually gives place soon to a whirling to the right.

The direction of motion after a chemical stimulus has thus in Oxytricha no evident relation to the localization of the stimulus.

Hypotricha in general. — Without determining the species, the reactions of a considerable number of other Hypotricha (using this group as limited by Bütschli) were examined. All agree in the main features of structure with Oxytricha, and the method of reaction was practically identical in all. At any stimulus, except a mechanical stimulus confined to the posterior half of the body, all the species examined jerk backward more or less, turn to the right, then go forward again. No exception to this turning to the right after a stimulus was seen in any case.

As shown by the foregoing description of Oxytricha and other Hypotricha, the infusoria of this group present in their reactions certain features which give much complication to their locomotion as a whole. (1) In following an irregular substratum, such as a crooked alga filament, the animals may turn to the right or left or up or down, — the direction being determined solely by the form of the surface on which they are moving. This may be a mechanical result of the method of locomotion. As is well known these animals use a number of large cilia or cirri on the ventral side like legs for creeping. In following an alga bent to the left, for example, on coming to the bend the cirri on the right side would on being pushed forward find nothing to rest upon; the left cirri on the contrary would find a point of support, and thus pulling alone on the body, would necessarily turn it to the left. (2) In the same species some of the unstimulated individuals follow a straight path; others a path which curves to the left; others a path which curves to the right. Since, so far as observed, the path of a given individual is always curved in the same direction, it seems probable that the curve of the path is due to the form of the body; but the fact that the degree of curvature in the path of a single individual varies indicates the possibility of the presence of other factors in determining the direction of movement.

In spite of this greater complication in the movements of the Hypotricha as compared with other infusoria, it is in this group that the reaction to a stimulus by turning in a definite direction (to the right) without regard to the position of the source of stimulation, is peculiarly striking and easily observed. This is of course owing to the fact that the Hypotricha do not revolve as they move through the water, as is done by most other infusoria, but run along the bottom with the ventral side below. There is thus no difficulty in determining the direction in which the animals turn after stimulation, and this is invariably toward the right side.

Other Infusoria. — The foregoing discussion contains an account of the reactions of representatives of the chief groups of the infusoria, with the exception of the Peritricha. The members of this group are nearly all fixed forms, and hence not fitted for determination of the questions raised in this paper. It is true that such forms as Vorticella frequently break from their stalks and swim freely through the water. But the movement seems less well coördinated than in the other infusoria, and to almost every stimulus these creatures respond by sharply contracting the body and folding the adoral cilia inward, at the same time of course ceasing to move. I have not as yet therefore succeeded in determining with certainty any such regularity in their motor reactions as was observed in other infusoria. In addition to the species whose reactions are above described, I have studied many other species which did not, for one reason or another, give clear results, and which were therefore not included in my account. It is of course possible that a complete analysis of the reactions of some of these forms may give results differing in principle from those here detailed; in other words it is possible that essentially different laws of reaction may obtain as between different infusoria. But from the considerable number of representatives of different groups whose reactions were worked out and above described, it is believed more probable that the general principles of the motor reaction methods for the entire group have been obtained. No attempt has been made in the present paper to give an exhaustive account of the reactions of any of the organisms studied; rather were their reactions examined with a view to the answering of certain definite questions, stated in the introduction.

We will now proceed to an analysis of the observations above recorded and a discussion of the conclusions to be drawn in regard to the nature of the motor reactions of these creatures.

IV. ANALYSIS OF THE OBSERVATIONS, SUMMARY, AND CONCLUSIONS.

(1) The first of the questions proposed in the introduction was, Has the given Protozoön a fixed formula for motor reactions, similar to that of Paramecium?

This question has now been answered for representatives of the main groups of Flagellata and Ciliata, — including a Flagellate with two flagella (Chilomonas), others with a single flagellum (Euglenidæ), representatives among the Ciliata of the Holotricha (Paramecium, Loxophyllum, Colpidium, Microthorax, Dileptus, Loxodes, and Prorodon), of the Heterotricha (Stentor, Spirostomum, and Bursaria), and of the Hypotricha (Oxytricha and others). In all these species a fixed and definite type of motor reflex was shown to occur, though with much variation in details. In general, when one of these organisms is affected by a decided stimulus, the motor reflex takes the form of a swimming toward one end (structurally defined), turning toward one side (structurally defined), then swimming again with the same end in front as before stimulation. This question is then to be answered in the affirmative, — under the limitations given in the following paragraphs.

In the details of the reflex there is much variation. This may be brought out by noting the difference in behavior when each organism comes, for example, against the edge of a drop of a weak solution of some chemical, as sodium chloride. The lowest motor reaction is shown perhaps by Euglena viridis, which simply turns toward a definite side (the larger lip): if stimulation continues, it takes the spherical form and prepares to encyst. There is here no swimming backward and no contraction. A still lower condition is found in Euglena spirogyra and E. oxyuris, in which there is no motor reaction at all, the organisms simply remaining quiet and beginning the process of encystment at any evident stimulus. Most of the organisms studied gave the typical motor reflex above characterized. In others

(Spirostomum and Stentor), a strong contraction of the body is an additional feature of the reaction.

(2) The second question proposed was, After stimulation does the organism always turn toward a certain structurally defined *side*, without regard to the nature or position of the stimulus?

This question is answered for all the organisms studied in the affirmative. In any given organism the side toward which the creature turns after a stimulus is invariable, and has no relation to the localization of the stimulus.

The side toward which the animals turn is variously defined. In very closely related forms, as in the different Hypotricha (Bütschli), common structural features define the direction toward which the animals turn (in this case, toward the right side). In more distantly related species it is not possible to name any single structural peculiarity by which the direction of turning can be defined. Thus Chilomonas turns toward its lower or smaller lip, Euglena toward its upper or larger lip. Paramecium, Colpidium, Loxodes, and Spirostomum turn toward the aboral side; Loxophyllum and Dileptus toward the oral side. Microthorax turns toward its "dorsal" edge; Stentor, Bursaria, and the Hypotricha toward the right side. Prorodon turns toward one side which is invariable, but not structurally marked. There is of course such great variation in the structure and form of these animals that the same term (as " right " or " aboral ") may have very different significations with respect to the general form in different species, so that there is no especial reason for expecting uniformity in this matter. The direction of turning in a given case depends on the structural and functional peculiarities of the locomotor organs, taken in connection with the geometrical form of the body on which they act.

The relation of the direction in which the animals turn after stimulation to the usual direction of locomotion is likewise variable. Most of these organisms, as they swim forward unstimulated, swerve toward one side or the other,.— so that a spiral course results, if they at the same time revolve on the long axis. This side toward which the creature swerves may or may not be the same as the side toward which it turns after stimulation. In Chilomonas, Paramecium, Microthorax, Dileptus, Stentor, Spirostomum, and Bursaria they are the same; in Loxophyllum and Colpidium the animals swerve in ordinary locomotion toward one side (owing to the form of their bodies), while after stimulation they turn toward the opposite side. The

Hypotricha may swerve from a straight line either to the right or to the left, but after stimulation the turn is always to the right.

(3) Before taking up the third question proposed in the introduction, it will be well to state briefly the facts brought out in regard to the comparative sensitiveness of different parts of the body. As shown by localizing mechanical stimuli, *the anterior end is much the most sensitive part of the body.* This was demonstrated for Paramecium, Dileptus, Loxodes, Stentor, Oxytricha, and in a less marked degree for Spirostomum; it is probably true for the others also.

(4) The third question proposed in the introduction was, After stimulation does the organism always move, before turning, toward a certain structurally defined *end* (the "posterior") without regard to the nature or position of the stimulus?

This question is answered, in the general form above stated, in the *negative.* Yet the conditions and limitations of this negative are such that an analysis of the observations is necessary for an appreciation of its signification.

(*a*) As pointed out in the accounts of Chilomonas and Paramecium, the usual *forward* motion of all the organisms above described may be considered a reaction to a stimulus, if the resting condition be taken as a starting point — inasmuch as the beginning of this motion is due to an external change. The resting individual may be induced to resume the forward motion by any very gentle movements such as will separate it from the object against which it is resting, without giving it a stimulus of a pronounced character. It then requires another stimulus to induce again the resting condition.

(*b*) Some forms, as Euglena viridis, do not swim backward at all even when strongly stimulated, — the typical motor reflex consisting merely of the turning toward a definite side.

(*c*) For other organisms, the following facts are brought out by the experiments with localized stimuli: —

In Dileptus, Loxodes and Oxytricha, the end toward which the animal moves after a mechanical stimulus depends upon the localization of the stimulus; to such a stimulus at the anterior end the animals react by swimming backward, while if the stimulus is at the posterior end they swim forward.

For chemical stimuli, on the other hand, in the same infusoria, as well as in others, the *absence* of any such dependence of the direction of motion on the position of the stimulating agent was demonstrated.

Since the experiments with localized mechanical stimuli clearly demonstrate that the organisms just named have the power to distinguish stimuli upon the posterior part of the body from those on the anterior part, and to vary their reaction accordingly, the question arises as to why this power is not exercised in the case of chemical stimuli. The fact that the organisms swim backward when stimulated by a chemical substance diffusing from the rear has cost the lives of many infusoria in these experiments. This perverse and useless method of reaction has been demonstrated to occur in Chilomonas, Paramecium, Loxodes, Dileptus, Spirostomum, Oxytricha, and other Hypotricha.

The explanation of this lack of appropriate reaction in the case of chemical stimuli is probably as follows: As stated above, it is proven in many cases that the anterior end is much more sensitive than the remainder of the body, — the latter being comparatively impercipient. Thus a light touch with the glass rod, that would at the anterior end induce a strong reaction in Paramecium, Loxodes, Dileptus, Stentor, or Oxytricha, produces in these same organisms when applied to the posterior part of the body no reaction at all. Now, suppose a chemical substance diffusing somewhere in the rear of one of these infusoria. As the very dilute solution first reaches the posterior end, it is too weak to act as a stimulus upon this comparatively unsensitive part. The animal therefore remains quiet, and the chemical continues to diffuse, until, coming from the rear, it reaches the sensitive anterior end. Thereupon a strong reaction is induced, which, resulting from a stimulus applied at the anterior end, takes the form of swimming backward, etc., rather than forward. The animal may thus enter the destructive solution and be killed by it.

Spirostomum ambiguum presents an exception to the general rule, in so far that even mechanical stimuli applied to the posterior end usually induce the swimming backward, exactly as when applied to the anterior end. The greater relative sensitiveness of the posterior end of Spirostomum and its habit of contracting strongly at any stimulus seem to be connected with this fact. (See the account of Spirostomum above.)

To unlocalized stimuli — that is, stimuli applied to the entire surface of the animal at once, as when they are dropped into some chemical solution or when the vessel containing them is strongly jarred — all the organisms respond by swimming first *backward*. In such a case it is perhaps only the anterior end that actually receives a stimulus.

In a recent paper[1] I have attempted to set forth the bearing of the results gained by a study of the motor reactions of Paramecium on the psychology of that animal. The investigations recorded in the present paper require a modification of one of the statements therein made, — if not for Paramecium, at least for other infusoria. This is the statement that the direction of motion in the motor reaction has no relation to the localization of the source of stimulus. This statement was based on the reactions of Paramecium and other infusoria to localized chemical stimuli, and the reactions of Spirostomum to localized mechanical stimuli. These phenomena taken alone seem to justify the statement for Paramecium and Spirostomum, yet the facts obtained by a study of other infusoria show that the statement cannot be generalized for this group of animals. We have in the infusoria a remarkable transitional stage toward a real perception of the localization of the stimulus, — reaction with regard to such localization so far as motion along the body axis is concerned; a blind reflex without regard to the localization of the stimulus, so far as motion sideways is concerned.

(5) On the whole, the investigation has shown that the motor reaction plan of the infusoria studied is essentially the same as that previously described for Paramecium. Until it is clearly shown that some members of these groups react in a manner essentially different from that which I have described, we may assume provisionally that this manner of reaction is characteristic for the Flagellata and Ciliata in general. We may therefore extend provisionally the chief general conclusions drawn from a study of the movements of Paramecium to the Flagellata and Ciliata as a whole. The more important of these conclusions may be stated briefly as follows: —

1. The motor reactions to stimuli in the Flagellata and Ciliata take the form of a reflex of definite character, the usual features of which are that the animal moves backward some distance, turns toward a structurally defined side, then moves forward.

2. Different kinds of stimuli do not produce correspondingly different kinds of reaction, but this motor reflex is produced by chemical stimuli of all sorts, by fluids active through their osmotic pressure, by heat, by cold, and by mechanical shock; in fact, by all agents capable of causing a motor reaction. Chemotaxis, tonotaxis, thermotaxis, etc., are therefore not essentially different forms of

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[1] JENNINGS: American journal of psychology, 1899, x, p. 503.

activity: they are due to the same reflex, merely induced by different agents.

3. The direction of motion throughout this reflex has to only a very limited degree a relation to the localization of the stimulus. The direction of turning has absolutely no relation to such localization, being determined by structural differentiations. Whether motion shall take place backward or forward along the body axis is however to a certain extent determined by the localization of the stimulus.

4. The general effect of this reflex is to take the organism out of the sphere of influence of the agent causing the reaction, and to prevent it from re-entering.

5. Chemotaxis is by no means a passive motion due to attraction or repulsion of the protoplasmic substance by other substances, but is an active movement due to the production of this motor reflex by the chemical agent in question. The organisms leave certain areas vacant (" negative chemotaxis ") as a result of the fact that the influences at work in these areas cause this motor reflex. They gather in certain areas (" positive chemotaxis ") when the conditions within these areas are *not* such as to cause the motor reflex, while the surrounding influences do cause it. There is then no such thing as direct attraction or repulsion shown by these animals. Corresponding statements may be made for thermotaxis, tonotaxis, etc.

6. The motor reflex through which these reactions are produced is of the same order as the motor reflexes of higher animals, so that there is no reason for holding the reactions of these unicellular organisms to be of an intrinsically different nature from those of higher forms.

7. The behavior of these organisms shows them to occupy an extremely low place in the psychological scale, most of their activities being due to a single reflex.

8. There is evidently no immediate analogy between the reaction movements of these unicellular organisms and the growth movements of higher forms (" tropisms "), so that the phenomena shown by the former do not justify the drawing of direct conclusions concerning the latter.

These conclusions, as well as others of a less general nature, have been developed in detail by the author in previous papers,[1] so that it is not necessary to dwell upon them here.

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[1] See, in addition to the papers previously cited, a lecture by the author on "The Behavior of Unicellular Organisms," in the Woods Holl Biological Lectures for 1899.

The investigations above recorded were carried on in the Laboratory of the U. S. Fish Commission for the Biological Survey of the Great Lakes at Put-in-Bay, Ohio, during the summer of 1899. I desire to acknowledge my indebtedness to the officials of the Commission and to Professor Jacob Reighard, Director of the Survey, for their courtesy and assistance throughout the work as well as for permission to publish this paper.

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STUDIES ON REACTIONS TO STIMULI IN UNICELLULAR ORGANISMS. — VI. ON THE REACTIONS OF CHILOMONAS TO ORGANIC ACIDS.

BY H. S. JENNINGS.

STUDIES ON REACTIONS TO STIMULI IN UNICELLULAR ORGANISMS. — VI. ON THE REACTIONS OF CHILOMONAS TO ORGANIC ACIDS.

By H. S. JENNINGS.

IN the January number of this Journal appeared two papers dealing with the reactions of infusoria.[1] Both the papers treat, among other matters, somewhat fully of the reactions of Chilomonas, but from a different standpoint in each case. Garrey's paper considers the subject chiefly from a chemical standpoint, giving an account of the chemical substances in solutions of which Chilomonas forms aggregations, and of other substances which the animals leave vacant, and determining the exact factors in the solutions to which these results are due. Garrey did not, however, work out the mechanism of the reactions, — that is, he did not determine the exact movements of the animals when stimulated. My own paper, on the other hand, dealt with the matter from the side of the organism, and was concerned especially with the mechanism of the reactions, showing the exact movements of the animals under stimulation. I did not, however, treat of the special chemical substances in which aggregations are formed, or which are left vacant, by the organisms. The direct application of the reaction mechanism which I have described to the phenomena of aggregation, etc., described by Garrey has, therefore, not been made. As the conception presented by Garrey of the nature of the activities of the organism in these reactions is different from that to which I was led by a study of the reaction mechanism, it seems important to make this application. I am convinced that there is no contradiction between the observations of Garrey on this point and my own observations, but that on the contrary the two supplement each other neatly and lead to the same conclusions.

The organism concerned is the small Flagellate *Chilomonas*. Brief descriptions of the organism are given in both the papers above-mentioned; one point which becomes of much importance for an understanding of the essential nature of the reactions was not specifically mentioned in either paper, however. This is the well-known fact that Chilomonas is an unsymmetrical animal, the so-called "upper," or larger lip lying at the dorso-dextral angle of the body, as figured by Bütschli, in Fig. 9 b of Plate XLV, in his great work on the Protozoa, — so that the organism cannot be divided either dorso-ventrally or dextro-sinistrally into similar halves, and it is impossible to speak of "symmetrical points on the surface of the body," as is done in bilaterally symmetrical organisms.

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[1] (1) The effect of ions upon the aggregation of flagellated infusoria, by Walter E. Garrey: This journal, iii, no. vi, pp. 291–315. (2) Studies, etc. V. On the movements and motor reflexes of the Flagellata and Ciliata, by H. S. Jennings: This journal, iii, no. vi, pp. 239–260.

Garrey finds that certain chemicals "cause the organism to become restless, very swift shooting movements being caused." As a result of these movements the organisms soon leave the area of operation of the chemical causing the reactions. This phenomenon Garrey calls "chemokinesis." Expressed in the more precise terms of the "motor reflex" described in my own paper (*loc. cit.*), this is as follows. These chemicals cause in a marked degree the motor reflex of Chilomonas; this reflex consists of the following activities. The animal darts *backward*, turns sideways *toward the smaller lip*, then swims forward in the new path thus determined by the position of the smaller lip. This being repeated, the animal is in time brought out of the region of the agency causing the motor reaction. The whole phenomenon is evidently precisely analogous to the conduct of Paramecium in leaving vacant a drop of some chemical to which it is "negatively chemotactic" (or chemokinetic). The only difference between the two cases seems to be that Chilomonas forms a ring around the outside of such a drop, while Paramecium does not. This is due to the fact that Chilomonas is normally much less active than Paramecium, and usually comes to rest as soon as a motor stimulus is lacking; it therefore comes to rest as soon as it gains the outside of the drop of the chemical which acts as a stimulus. Paramecium, on the other hand, is a strong swimmer, and once in motion, it continues to move until stopped by another stimulus; it therefore forms no collection about such a drop. — The above is given as merely supplementary to Garrey's account, and not as in any sense a correction of it: I apprehend that there would be no disagreement on these points.

In certain organic acids (acetic, butyric, lactic) and their salts Garrey finds that Chilomonas forms dense aggregations, — as Paramecium does in weak solutions of acids of any kind. The special problem here is, how do the organisms gather in the region of the organic acid? Garrey states that "a study of the mechanics by which the organism is oriented, or by which it is prevented from moving from the ring into the stronger acid of the clear area or the weaker acid surrounding the ring, proved fruitless" in his case, so that the question as to the mechanics of the phenomenon remains an open one. In what follows, I shall set forth observations which I believe give a clear answer to this question.

In studying the reactions to organic acids (acetic and butyric were chiefly used), I have employed two methods. In one of them an apparatus similar to that described by Garrey[1] was used. This method, in which the reactions are examined in a chamber 1 to 1½ mm. deep, is well suited for showing the grosser phenomena of aggregation, since it is thus possible to work with a very large number of organisms, and the aggregations formed are dense. On the other hand, it is, of course, impossible to study the exact behavior of creatures but 40 μ long in a layer a millimetre deep. The second method was that used by me in studying the reactions of Paramecium: it is described and figured in the second of my Studies,[2] p. 314. In this method the animals are mounted in a very thin layer of water under a supported cover-glass, and a drop of the substance to be tested is introduced with a capillary pipette into the preparation. The aggregations of the animals are then produced in the same manner as by the first method, but of course contain comparatively few individuals, so that the gross appearance is not striking. But the thickness of the layer of water can be diminished to any desired extent by decreasing the size of the supporting glass rods, so that the individuals can be watched even with a high power lens; thus the exact behavior of the animals may be noted.

Using this second method, when a drop of weak acetic or butyric acid is introduced, the swimming Chilomonads behave as follows. Those which swim against the edge of the drop enter it without reaction. If they continue to swim across it, when they come to the opposite boundary, where they would, if unchecked, pass again to the outer medium, the change in the solution at this point produces the characteristic reflex, by which the animals are turned toward the smaller lip, and thus must pass back again into the drop. This may continue, so that the animal remains in the drop. Others enter in the same way and are retained in the same way, so that the drop in time contains large numbers of individuals. Frequently an individual on entering the drop simply settles down against the slide or cover-glass, so that no motor reaction or orientation of any sort is necessary to keep it in the drop. The reason why a number of Chilomonads collect in the drop of acetic acid is then as follows. When the swimming individual comes from the outside against the drop of acid, the change in passing from the outer medium into the drop is not of such a nature as to cause a motor reflex, while *the opposite change, from the acid to the outer medium, is of such a nature as to produce the motor reflex.*[1] The further question, of why one sort of change should cause a motor reflex, while the opposite does not, is of course here, as in other motor reflexes, unanswerable; to determine what agencies will cause a motor reflex is always a matter for experiment.

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[1] GARREY: *loc. cit.*, p. 294, Fig. 3

[2] JENNINGS: This journal, 1899, ii, pp. 311–341.

As to the matter of orientation, it is, of course, evident that when a Chilomonas enters the drop, it will, as a rule, be so oriented that its anterior end is directed approximately toward the centre of diffusion of the chemical, otherwise it would not enter the drop at all. If the drop in diffusing reaches a number of resting Chilomonads, these start to swim; those which swim toward the centre of the drop are not stopped, while those which swim in other directions, coming quickly to the edge of the drop, give the motor reflex, and are therefore turned back into the drop. At a certain time then, under these circumstances, if the drop is large a considerable number of Chilomonads may be seen swimming with common orientation toward the middle region of the drop, since motion in any other direction is quickly stopped by the production of the motor reflex. Under ordinary circumstances, however, neither the individuals in the drop nor those around it will all show at a given instant a common orientation. Each organism is in a certain sense oriented whenever the motor reflex occurs, in so far as it cannot swim farther in the original direction, but may swim in some other direction. But the motor reflex does not usually occur in a large number of individuals at the same time, and it is only when this occurs that a common orientation of a large number of individuals can be expected. In spite of this usual lack of common orientation, the Chilomonads do in time form marked aggregations in the drops of acid, in the manner I have described.

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[1] This is perhaps the most important point for understanding how the collections are formed: if it is not clearly apprehended the remainder of the account cannot be understood.

In the preparations 1 to 1½ mm. thick, containing, of course, an immensely greater number of individuals coming under the influence of the stimulus at once, the Chilomonads about the diffusing solution do show a common orientation, according to Garrey : " It is easy to see that the organisms within this area are oriented with anterior ends (those bearing flagella) directed toward the ring, and that they are swimming in strictly radial lines, the lines of diffusion, toward the diffusing drop."[1] This is Garrey's ground for assuming that the aggregations are not brought about through a motor reaction such as I have described; he states that, on the contrary, these aggregations are due to " true chemotropism." As to the nature of chemotropism, he quotes with approval Professor Loeb's generalization, " The essence of chemotropic orientation would then consist in the animals placing themselves in such a position that symmetrical points on the surface of the body are cut by the diffusion lines at the same angle."

Now, in interpreting Garrey's statement as to the orientation of Chilomonas, it is necessary to recall two points not mentioned by Garrey. The first is that Chilomonas is unsymmetrical, so that no " symmetrical points on the surface of the body " are distinguishable, and second, that the animal swims in spirals of some width, and not in *straight* radial lines. The statement as to their orientation implies then only that their anterior ends are directed in general toward the centre of diffusion, — the lack of symmetry and the spiral path making any more precise statement impossible.

In the cells 1 to 1½ millimetres deep that Garrey used the number of organisms in a given area is enormously greater than in the preparations thus far discussed, and they are not confined to a thin layer, so that it is comparatively difficult to observe individuals. Now when the diffusing acid reaches a large number of organisms, they at once start to swim in various directions. Those which swim away from the centre of diffusion quickly come to the outer boundary[2] of the acid and are turned through the motor reaction back into the acid area; only those which swim approximately toward the centre of diffusion (therefore in radial lines) continue on their course unhindered. Hence shortly after the beginning of the reaction a large number of animals are seen swimming toward the centre of diffusion, none in the opposite direction, while a certain number are swimming in scattered directions. Now, of course, when out of a confused movement of a large number of particles there suddenly arises a movement of a considerable proportion of the particles *in a definite direction*, the attention is immediately attracted to the definite movement, and it is only by a strong effort that the eye can be brought to attend to the individuals that are swimming irregularly in all directions. It would be necessary for me to state that my observations did not agree with those of Garrey, if by his statement that "the organisms swim toward the centre of diffusion along radial lines" he meant that all or approximately all the organisms swim in this manner. Certainly no one would make such a statement for the aggregations which occur when a drop of the acid is introduced into a preparation of the animals in a thin layer of water, where the movements of the individuals can be observed. Garrey does not state that *all* the organisms swim in radial lines, nor does he give any idea of the proportion that does so. Indeed, he gives distinctly the impression that this precise common orientation is not, as a rule, very marked (which would agree with my observations) when he says that "this migration [along radially disposed lines toward the centre of the diffusing drop] was most markedly evident in *two or three*[1] experiments in which the organisms were gathered very densely about débris situated some distance from the mouth of the diffusion tube. As soon as they came under the influence of the diffusing acid the organisms left the débris and fairly swarmed into the acid."[2] This observation, of course, accords perfectly with the account I have given of the way orientation takes place. Coming under the influence of the stimulus, the large number of organisms about the débris at once begin to swim (owing to Garrey's "chemokinesis," or my own "reaction to a weak stimulus").[3] But only those which swim toward the centre of diffusion can continue their course; others come quickly against the boundary of the fluid and are stopped and turned (through the motor reflex) till they too finally come into a direction approximately toward the centre. Thus in a moment after the beginning of the reaction a stream of organisms is seen passing toward the centre of diffusion, as Garrey has described. (See the analogous phenomena in the case of Paramecium in thermotaxis.[4])

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[1] *Loc. cit.*, p. 297.

[2] By "outer boundary" I mean, of course, in every case that point where the decrease in the concentration of the chemical is sufficient to be perceptible to the organism, — *i. e.*, sufficient to cause a reaction. This "boundary" is, of course, not a sharp line; it varies for different organisms.

[1] Italics mine.

[2] *Loc. cit.*, p. 310.

[3] Studies, etc., V. (*loc. cit.*), p. 232.

[4] Studies, etc., II. (*loc. cit.*). p. 335.

It seems to me, therefore, that there is no disagreement between Garrey's observations and my own, in this matter. Chilomonas collects in regions of weak acetic or butyric acid through the agency of the motor reflex described in the fifth of my Studies, in a manner exactly analogous to the collections of Paramecia in drops of acid, described in the second of my Studies.[1]

Whether these phenomena should be called chemotropism, or chemotaxis, or chemokinesis (the term proposed by Garrey), of course depends on the definition to be given to these words. I have hitherto used the name (chemotaxis) which was employed before the exact character of the phenomenon was known, preferring to let nature supply the definition. I find it difficult to determine whether the method of reaction described above falls within the definition of chemokinesis given by Garrey or not.

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[1] *Loc. cit.*, p. 315.

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STUDIES ON REACTIONS TO STIMULI IN UNICELLULAR ORGANISMS. — VII. THE MANNER IN WHICH BACTERIA REACT TO STIMULI, ESPECIALLY TO CHEMICAL STIMULI.

By H. S. JENNINGS and J. H. CROSBY.

[From the Zoölogical Laboratory of the University of Michigan, Jacob Reighard, Director.]

STUDIES ON REACTIONS TO STIMULI IN UNICELLULAR ORGANISMS.—VII. THE MANNER IN WHICH BACTERIA REACT TO STIMULI, ESPECIALLY TO CHEMICAL STIMULI.

[*From the Zoölogical Laboratory of the University of Michigan,*
JACOB REIGHARD, Director.]

BY H. S. JENNINGS AND J. H. CROSBY.

IN earlier numbers of this series of studies the manner in which ciliate and flagellate infusoria react to various stimuli has been described. It has been shown, especially in the second,[1] fourth,[2] and fifth[3] of these papers, that the so-called tactic phenomena of these organisms are not as a rule due to an orientation, or direct turning of the organism to or from the source of stimulus, as has often been assumed to be the case. On the contrary, the phenomena are due to a definite movement or reflex action, produced by the stimulating agent, and always taking place in essentially the same manner. The organisms when stimulated by a chemical, by heat, by cold, by mechanical shock or other similar agent, swim backward and turn toward a structurally defined side. To this simple reaction is due the collecting of the organisms in certain regions and their apparent avoidance of other regions, — the so-called positive and negative chemotaxis, thermotaxis, etc. (The reaction to the electric current is complicated by other factors).

Early in the progress of the work, it was incidentally observed in a number of cases that the bacteria have an analogous method of reaction. At that time opposition had developed in certain quarters to the account given in these studies of the method by which the so-called tactic phenomena take place in the infusoria. In view of this fact, and the further fact that Pfeffer in his classical studies on the reactions of unicellular organisms had distinctly asserted[4] that the reactions of the bacteria were not of the nature which our observations showed them to be, it was not deemed worth while to publish an account of these reactions of the Bacteria until the description given of the reactions of the infusoria had been confirmed. This description has now been confirmed and extended to other organisms by various observers. A careful study of the movements and reactions of certain bacteria was therefore undertaken during the past winter, with the intention of publishing the results of the investigation.

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[1] This journal, 1899, ii, pp. 311–341.

[2] *Ibid.*, pp. 355–379.

[3] *Ibid.*, 1900, iii, pp. 229–260.

[4] "Dass die Ansammlungen nicht etwa nur zu Stande kommen, weil die zufällig einschwärmenden Organismen beim Versuch des Entfliehens zurückschrecken, lehrt die direkte Beobachtung." —Pfeffer, Untersuchungen aus dem botanischen Institut, Tübingen, 1888, ii, 648, note. See also ibid., 1884, i, 464.

This work was entirely finished when the valuable paper of Rothert,[1] dealing partly with the same subject, appeared. This paper, so far as it covers the same ground, agrees throughout with our own observations and clearly establishes the fact that the reaction method of the bacteria to chemicals is not by an orientation, but is analogous to that of the infusoria, as described in these studies.

As the subject is of much interest, and as our work was done from a different standpoint from that of Rothert, with different methods and, to a certain extent, different organisms, a brief account of our own observations will not be superfluous even after the publication of Rothert's paper. In a field where so much uncertainty and disagreement exists, the mutual confirmation of two investigators working independently is of importance. For a discussion of the literature and of the general bearing of the results, reference should be made to the paper of Rothert.

The bacteria studied by us were those occurring in cultures of hay and of aquatic plants decaying in water. Two species of Spirillum, apparently Spirillum volutans and S. undula, were selected for special investigation.

The gross features of the reactions of bacteria to chemicals, as usually shown, are well known through the work of Engelmann,[2] Massart,[3] Verworn,[4] and others. Particularly striking is the reaction to oxygen. The bacteria, mounted on a slide and covered with a cover glass, collect (1) about bubbles of air; (2) about the edge of the cover glass, next to the air; (3) about green plant cells, diatoms, desmids, etc., which are giving off oxygen through the action of the chlorophyll. (See in Verworn's General Physiology, Figs. 211 and 214, and Davenport's Experimental Morphology, Part 1, Figs. 3–5*a*.) Such collections may be observed in any preparation containing the ordinary bacteria of decay.

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[1] ROTHERT: Flora, 1901, lxxxviii, pp. 371–421.

[2] ENGELMANN: Archiv für die gesammte Physiologie, 1881, xxv, pp. 285–292.

[3] MASSART, J.: Bulletin de l'Académie royale de médecine de Belgique, 1891 (3), xxii, pp. 148–167.

[4] VERWORN: Psychophysiologische Protistenstudien, 1889, pp. 103–106.

How are these collections formed? Do the bacteria turn and direct their course toward the centre of diffusion of the oxygen,— proceeding directly toward the region of greatest oxygen density? Or are the collections brought about more indirectly, in a manner similar to that by which Paramecium collects in regions containing an acid?

Before describing the observations by which this question is answered, it will be necessary to give a brief account of the form and usual movements of the organism Spirillum.

Spirillum volutans forms an elongated rod, very slender, with a length of from 15 to 50 μ. It is curved into the shape of a spiral of from two to six turns, so that it resembles a corkscrew in form. At each end of the spiral are found one or two flagella. There is no observable difference between the two ends of the organism, nor is there any marked differentiation of two sides, such as distinguishes oral and aboral sides in Paramecium, for example.

Movements.— Spirillum swims in the direction of the long axis of the spiral by means of its flagella. At the same time it revolves, the revolution following the direction of the spiral, and being therefore (usually at least) from left over to right, if one faces in the direction in which the organism is swimming. At intervals the movement is reversed, the organism swimming then with the opposite end in advance, and revolving in the opposite direction. As a rule, neither end seems to be preferred as the anterior one, Spirillum swimming indifferently in either direction. Given individuals are observed, however, at times to swim for long periods with a certain end in advance, the reversals lasting but a moment.

Occasionally an individual may be seen revolving on its long axis without progressing in either direction, while in other cases there is a rapid whirling on a tranverse axis. But these methods of movement are rare.

"**Chemotaxis.**" —If the Spirilla are mounted on a slide beneath a cover-glass, in company with some desmids or other green algal cells, after a time they will be observed to have formed collections about the algæ, as illustrated in the figures referred to above. How does this occur?

Careful observation shows the course of events to be as follows: —

At the beginning the bacteria are scattered uniformly throughout the preparation. They are swimming rapidly in all directions. At first they pass close to the green plant cells without any reaction whatever. The algæ begin, in the light, to give off oxygen, so that after some time each desmid or other alga must be conceived as surrounded by a zone of water impregnated with oxygen.

Now begins the collection of the Spirilla about the algæ. The bacteria surrounding the algæ do not change their direction of motion and swim toward the centre of diffusion of the oxygen. On the contrary, all continue to swim in the same direction as before. A Spirillum passing close to the alga into the oxygenated zone does not at first change its movement in the least. It swims across the zone till it reaches the other side. It is here that the reaction occurs; the organism reverses its movement and swims in the opposite direction till it reaches the opposite boundary of the oxygenated area. It then reverses again, and this is continued, — the direction of movement being reversed as often as the organism comes to the boundary of the zone of oxygen. The Spirillum therefore remains within the area, which thus acts like a trap. Other Spirilla, swimming at random, enter the area in the same way, react at the outer edges in the same manner, and remain. In the course of time therefore the zone of oxygen swarms with Spirilla.

There is thus no orientation shown either by the organisms within the area or by those outside. *Within*, the Spirilla are swimming in all directions, crossing each other's paths at every angle, and agreeing only in the fact that the movement is reversed on coming to the boundary of the zone of oxygen. A single individual may be seen to oscillate back and forth from one side of the area to the other an indefinite number of times. *Without*, movements are occurring absolutely without relation to the position of the alga and its oxygen zone. Many Spirilla pass close to the edge of the zone, but do not enter unless their original course carries them directly into it. Many of the bacteria therefore remain scattered throughout the preparation, not gathering about the algæ, no matter how long the slide is allowed to stand. But through their continued movement in all directions, dense groups are soon formed about the algal cells.

It is evident therefore that the collections of bacteria arise through the agency of a "motor reflex" essentially similar in character to that of the infusoria, described in previous numbers of these studies.

This motor reflex consists in the bacteria in a reversal of the direction of movement, upon stimulation. The direct cause of the reaction is a change in the nature of the surrounding medium. In the cases already described, it is the change from water containing *much* oxygen to water containing *little* oxygen. The "boundary" of the oxygen zone, above spoken of, is of course merely the region where the change in oxygen density is sufficiently great to cause the reaction.

The bacteria collect in exactly the same manner about air bubbles, and about the edge of the cover-glass, next to the air. In these cases the bacteria usually collect in a narrow zone a short distance from the air surface. If their movements be observed here, it will be found that the reversal of motion is brought about in two different regions. (1) The passage from the optimum zone of oxygen to a region having *less* oxygen pressure causes the reaction. (2) Passage from the optimum into a region having *greater* oxygen pressure, — next to the air surface, — causes the reaction with even greater precision than the opposite change. The Spirilla therefore remain in the narrow optimum zone a short distance from the bubble or the edge of the cover-glass.

The above phenomena are cases of what has been spoken of as "positive chemotaxis." "Negative chemotaxis," or the avoidance of regions containing certain chemicals, takes place in the same manner, save that the reaction occurs when the organism comes, from the outside, against the outer boundary of the area in question. Thus, if a drop of a ½ per cent solution of sodium chloride be introduced beneath the cover-glass by means of a capillary pipette, the following phenomena will be observed. The bacteria do not orient themselves and move in radial lines away from the centre of diffusion of the salt solution. On the contrary, all move in random directions, as before. But on coming against the outer boundary of the salt solution, the organism reacts by reversing the direction of its movement. Hence it does not enter the drop. As every Spirillum that comes in contact with the drop reacts in the same way, the drop remains empty.

Solutions of most acids, alkalies, and salts act in the same manner, so that a drop of any of them (of sufficient concentration) remains empty when introduced beneath the cover-glass.

In addition to the observations on Spirilla, the reactions of a number of the other bacteria found in decaying vegetable matter were studied. In every case the reactions took place in the manner above described for Spirillum, so that there can be no doubt that this method is of general occurrence among the bacteria. In this respect our results agree throughout with those of Rothert.[1]

The same method of reaction often occurs when the bacteria strike against a solid obstacle. The movement is reversed, the organism swimming in the opposite direction.

This manner of reaction was first observed by Engelmann in the reaction of Bacterium (Chromatium) photometricum to light.[2] If a small circumscribed area on the slide is lighted from beneath, the bacteria, swimming at random, pass into this area in the same manner as described above for an area of oxygen. On attempting to pass from this light area into the dark, this organism, according to Engelmann, suddenly reverses its movement and swims backward, — thus remaining in the lighted area. This reversal lasts in the case of Bacterium photometricum but a short time, the organism beginning soon to swim forward again. This is due to the fact that this bacterium has flagella only at one end and normally swims with that end in advance. The reversal of the movement is therefore soon followed by a return to the original direction. This reaction was called by Engelmann a "Schreckbewegung;" it is clearly identical with the "motor reflex" described in these studies.

Bacteria thus react to chemicals, to mechanical obstacles, and to light (or darkness) in the same way, — by a "motor reflex," comparable to that of the ciliate infusoria.

This method of reaction is denominated by Rothert *apobatic* taxis, in contradistinction to *strophic* taxis, which consists in a turning of the organism toward or from the source of stimulus, so as to bring the axis of the body into a definite orientation with respect to the stimulus. The bacteria would thus show apobatic chemotaxis and apobatic phototaxis, using this method of denomination. The really fundamental phenomenon in these cases is the definite reflex action produced by the stimulus; whether aggregation or scattering of the organisms occurs and where it occurs, depend merely on what agencies produce this reflex.

The "motor reflex" of the bacteria differs from that of the infusoria in the same way that the form and structure of the body differ in the two cases. In such bacteria as Spirillum there is no differentiation as between the two ends, or between the two sides of the organism. In correlation therewith, movement takes place indifferently in the direction of either end, and the motor reflex consists merely of a reversal of the direction of the movement, — without subsequent return to the original direction except as a response to a new stimulus. In the infusoria there is a differentiation both between the ends and between the sides of the animal. The movements reflect these differentiations. The organism swims normally with a certain end in advance, and usually swerves toward a certain side. The motor reflex consists in a reversal of the direction of movement, so as to swim toward the opposite end, together with a turning toward a definite side, and this is always followed soon by a return to the original motion with the anterior end in front. In the case of Engelmann's Bacterium photometricum we have an interesting intermediate condition. Here there is a differentiation between the two ends of the organism, only one bearing flagella, while apparently all sides are alike. The reaction to a stimulus consists in a reversal of the direction of movement, as in the other bacteria, but without any turning toward a certain structurally defined side, such as occurs in the infusoria. But the reaction is followed, as it is in the infusoria, by a return to the original direction of movement. The reactions thus give throughout, in their simplicity or complexity, a faithful reflection of the structure.

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[1] *Loc. cit.*

[2] ENGELMANN: Archiv für die gesammte Physiologie, 1883, xxx, 95–124.

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STUDIES ON REACTIONS TO STIMULI IN UNICELLULAR ORGANISMS. VIII. — ON THE REACTIONS OF INFUSORIA TO CARBONIC AND OTHER ACIDS, WITH ESPECIAL REFERENCE TO THE CAUSES OF THE GATHERINGS SPONTANEOUSLY FORMED.

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STUDIES ON REACTIONS TO STIMULI IN UNICELLULAR ORGANISMS. VIII. — ON THE REACTIONS OF INFUSORIA TO CARBONIC AND OTHER ACIDS, WITH ESPECIAL REFERENCE TO THE CAUSES OF THE GATHERINGS SPONTANEOUSLY FORMED.

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IT is well known that when certain infusoria are left undisturbed they do not remain scattered, but gather in more or less dense groups. Thus, if they are mounted on a slide in a thin layer of water, soon dense aggregations will be formed in certain areas, while the remainder of the slide will be nearly deserted. One of the first investigators to describe this phenomenon was Pfeffer.[1] He observed its occurrence in Glaucoma scintillans, and less markedly in Colpidium colpoda, Stylonychia mytilus, and Paramecium. Pfeffer was inclined to believe that these aggregations were due, partly at least, to a contact stimulus, resulting from a striking of the organisms against small solid bodies, and especially against each other.

In the first of this series of studies,[2] this phenomenon in the case of Paramecium was subjected to a thorough examination. It was demonstrated that while the contact stimulus plays a certain part in the production of these aggregations, the chief factor involved is a reaction to carbon dioxide. The Paramecia tend to gather into regions where the water is impregnated with this substance. Since the animals themselves produce carbon dioxide in their respiratory processes, any spot where a few have gathered (owing to the contact stimulus or for any other reason), becomes a centre for the production and diffusion of this substance. Therefore other Paramecia collect here; more carbon dioxide is produced; more Paramecia collect, and in time a dense aggregation is formed. It was farther shown that this effect of carbon dioxide is due to the fact that it forms in water an acid solution ("carbonic acid") — the Paramecia collecting in the same way in any substance having a weakly acid reaction.

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[1] PFEFFER: Untersuchungen aus dem Botanischen Institut, Tübingen, 1888, ii, p. 618.

[2] JENNINGS: Journal of physiology, 1897, xxi, pp. 258–322.

As the other infusoria which form similar aggregations of course likewise produce carbon dioxide in their respiratory processes, it seems very probable, as was pointed out in the paper just referred to, that the same factors are at work here as in the case of Paramecium; that the spontaneous aggregations formed are due to the tendency of the organisms to collect in carbonic acid. This probability has been set forth also by later investigators, as for example in the recent paper of Rothert.[1] But no one has hitherto undertaken to determine by experiment in how far this may be true.

This is the problem which the study here presented has attempted to solve for a certain number of infusoria (sixteen species). The primary question to be answered is therefore as follows: — Are the spontaneous aggregations formed by certain species of infusoria due to their gathering in carbon dioxide excreted by themselves? The investigation involved a test of the reactions of the organisms studied both to carbon dioxide and to acids in general, and at the same time brought out a number of points as to the method in which the reactions of the organisms are produced; these secondary matters are likewise set forth briefly in the following paper.

METHODS.

The method of experimentation most used was that described in the first and second[2] papers in this series of studies. The organisms were studied in a thin layer of water, by mounting them on a slide covered with a large cover glass supported near its ends by slender glass rods. Their reactions were tested by introducing with a capillary pipette a drop of the substance in question beneath the cover glass, or in some cases by allowing it to diffuse inward from the side of the cover glass. In the case of gases, as carbon dioxide, it was found very convenient to proceed as follows. The gas is introduced into a large rubber bulb, such as is used with syringes or atomizers. To this is attached by the rubber tube a glass tube drawn out to a fine point. By inserting the point beneath the cover glass and pressing the bulb, a bubble of gas is introduced into the preparation. Where still different methods were used, these are mentioned in the account of results.

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[1] ROTHERT: Flora, 1901, lxxxviii, p. 402.

[2] JENNINGS: This journal, 1899, ii, pp. 311–341.

In attempting to determine the reactions of organisms to carbon dioxide, it is of course absolutely necessary that there should be no considerable quantity of this substance already present in the water. And since the organisms are continually producing carbon dioxide in appreciable quantities, some method of getting rid of the gas is a practical requirement of the highest importance. The simplest method is to aerate the water thoroughly immediately before each test. This may be done as follows. Place a few drops of the fluid containing the organisms, — as much as will be placed on the slide at once, — in a watch glass; then with a clean pipette inject it repeatedly over the surface of the watch glass, force bubbles into it, and mix it thoroughly with the air. Then place on the slide, cover, and perform the tests at once. Repeat the aeration before every test, as it requires only a very short time for water crowded with organisms to become impregnated with carbon dioxide. Of course it is not reasonable to expect organisms to gather in carbon dioxide when the water in which they are found already contains this substance in the optimum concentration. This precaution is equally necessary in testing other acids, as it is the common factor in all acids to which the effects of the carbon dioxide are due.

This or an equally efficacious method of aeration is an absolute necessity, if clear cut and constant results are to be obtained with carbon dioxide or acid solutions in general. This cannot be too much insisted on. Sometimes definite reactions will be obtained without aerating the water, in case it happens not to be already impregnated with carbon dioxide, but a little later the same organisms may give negative results.

A second precaution worthy of mention is the necessity of having the water containing the organisms relatively free from débris: — filamentous bacteria, and the like. Most of the infusoria are markedly thigmotactic, tending to come to rest upon coming in contact with small solid bodies. If a preparation contains a network of fine bacterial filaments, frequently the infusoria will not gather in the acid at all, but remain at rest on the filaments, while if the filaments are removed, as by straining through coarse cloth, marked positive reaction is at once obtained.

No attempt was made to determine quantitatively the exact strength of solution to which the organisms react. The purpose of the work was to determine whether the organisms do or do not give at any concentration a certain reaction to the substances in question. This was accomplished by beginning with a concentration so slight that the organisms did not react to it at all, and gradually increasing the strength till the solution is destructive. Somewhere between these limits will be found the characteristic reaction of the organisms. The value of quantitative determinations of the exact concentrations of acids to which the organisms react is largely illusory, in the majority of cases, as this varies with the amount of carbon dioxide present in the water, — a factor not under exact control. It varies also apparently with organisms from different cultures, and with the thickness of the layer of water in which the infusoria are confined. In experimenting with carbon dioxide especially, it is impracticable to attempt the use of solutions of known strengths; the introduction of a bubble of gas into the preparation gives all concentrations, from saturation next to the bubble to zero at some distance from it.

In the following account of the work, the organisms will be taken up in the order suggested by the nature of the results obtained.

A. ORGANISMS WHICH COLLECT IN SOLUTIONS OF CARBONIC AND OTHER ACIDS.

Chilomonas paramecium. — This small flagellate is perhaps the commonest and most abundant member of the group to which it belongs. It is therefore the most accessible form for experimentation on the Flagellata, and it will probably usually be employed when work on this group is undertaken. It is therefore important that the fundamental facts as to its reactions should be well established. An extensive piece of work has already been done by Garrey[1] on the reactions of this organism to chemicals, especially to acids. To our great regret we were compelled to come to results essentially different in some respects from those set forth by Garrey. It is unfortunate that there should be such disagreement, as this is likely to result in leaving the subject doubtful in the minds of other investigators. We believe however that we are able to point out exactly the factor to which the differing results are due, and to show that Garrey's results would probably not have differed from our own if this factor had been taken sufficiently into consideration. This factor is the normal presence of an acid, — a solution of carbon dioxide excreted by the organisms, — in the fluid in which Chilomonas occurs.

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[1] GARREY: This journal, 1900, iii, pp. 291–315.

Reaction to carbon dioxide. — Water containing Chilomonas is aerated in the manner above directed, and any bacterial filaments are removed by straining through coarse cloth. It is then placed on a slide, covered, and a bubble of carbon dioxide introduced. At first there is no gathering of the organisms, but soon they begin to collect about the bubble of gas, and gradually a dense ring is formed. Fig. 1 gives the general appearance of the progress of the experiment; it was taken from an actual preparation.

This experiment we have repeated many times, always (when the conditions were properly fulfilled) with the same results. The experiments succeed equally well in the apparatus used by Garrey, and figured on page 294 of his paper. With a long capillary pipette a bubble of carbon dioxide can be introduced into the chamber beneath the cover glass. The flagellates at once gather about it in a dense ring, while they do not thus gather about bubbles of air similarly introduced.

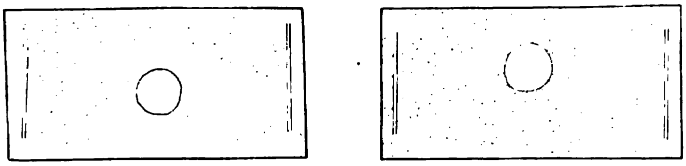

FIGURE 1, *a*. FIGURE 1, *b*.

FIGURE 1. — Reaction of Chilomonas to a bubble of CO_2. *a*, Preparation immediately after the introduction of the bubble, before the organisms have collected. *b*, The same preparation a few minutes later, showing the dense collection of infusoria about the bubble.

Chilomonas thus gathers about bubbles of carbon dioxide in dense collections, just as Paramecium does. The conditions above referred to as necessary of fufilment are (1) that the carbon dioxide should be properly removed from the water just before making the experiments; (2) that the bacterial filaments and other débris in the water should be largely removed.

The justification of the first condition is at once seen. It is idle to test the organisms with carbon dioxide when they are already immersed in a solution of that substance. It is undoubtedly to a neglect of this precaution, which is nowhere so much as referred to by Garrey, that the negative results of this investigator are due.

The necessity for the removal of the débris is evident on examining the behavior of the organisms. Chilomonas is very strongly thigmotactic; if when swimming through the water it comes in contact with a bacterial filament or bit of débris of any sort, it at once attaches itself by one of its two flagella, and comes to rest. Thus, in a preparation containing such filaments, all the individuals will soon be found quietly attached, which of course prevents their collecting anywhere.

Reaction to other acids.—To what factor is the collecting in the solution of carbon dioxide due? Is it, as in the case of Paramecium, due to the acid qualities of this solution (to the H ions, according to the dissociation theory)? To answer this question, tests were made with other acids, and the organisms were found to collect in weak solutions of these exactly as in the carbon dioxide solution. These results were clear cut and unmistakable; they were obtained with hydrochloric, nitric, sulphuric, acetic, formic, butyric, propionic, citric and oxalic acids.

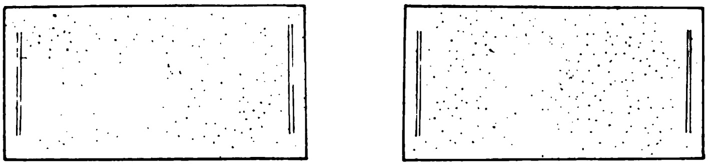

FIGURE 2, *a*. FIGURE 2, *b*.

FIGURE 2.—Reaction of Chilomonas to a drop of $\frac{1}{50}$% HCl. *a*, Preparation immediately after the introduction of the drop (no organisms either within or gathered about the drop). *b*, The same preparation a few minutes later.

As a control, the organisms were tested with distilled water; no gathering was formed about or in it at any time, but the organisms remained quite neutral in their behavior toward it.

The details of the phenomena vary with the strength of the acid solution used. With a stronger solution (say $\frac{1}{50}$ per cent HCl), the animals gather in a dense ring about the margin of the drop, leaving vacant the area within, containing the stronger acid (Fig. 2). With a weaker solution the organisms gather into the interior of the drop, leaving no part of it vacant (Fig. 3).

No characteristic difference was to be observed between their behavior to inorganic acids, such as HCl and HNO_3, and that toward organic acids, such as acetic and butyric, save that of course different concentrations were required to produce the same result. In Garrey's work, the results obtained with inorganic acids differed from those obtained with certain organic acids. Garrey studied the organisms in a much thicker layer of water, and introduced the acid through a tube-like opening at one side of the preparation. He observed that a dense gathering was formed about hydrochloric acid, but explained this as follows: When the strong acid reaches the organisms they begin at once to swim violently. This soon takes them outside of the area of acid, leaving it clear. On reaching the outer boundary, they stop, since there is no further cause for movement, thus forming a dense aggregation just outside the drop. "That in the zone surrounding the area there is a dense gathering (in other words that there is a ring formation) is in my opinion due to the fact that those individuals which were in the clear area are now gathered in the space immediately surrounding it" (*loc. cit.*, p. 296). The result is thus in a sense accidental, and would occur with any chemical which had the property of setting the organisms in violent movement. In Garrey's experiments no gathering was ever formed in the centre of a drop of inorganic acid, no matter how weak it was.

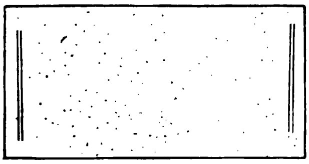

FIGURE 3.

FIGURE 3. — Collection of Chilomonas within a drop of $\frac{1}{100}\%$ HCl.

In our own work, the gatherings which occurred in drops of inorganic acids, were clearly not explicable in this manner. (1) When the drop was first introduced (Fig. 2 *a*) there were no individuals either within the drop or forming a ring about it. Later a dense ring was slowly formed (*b*). As there had been no individuals within the drop, of course the ring was not formed by individuals moving out of the drop and then stopping. Moreover, the process of ring formation is clearly evident to observation ; it is due to the swimming of organisms into the ring from outside. (2) If a weak solution of acid is used, it is at first empty, but later becomes completely filled with organisms (Fig. 3). This of course could not possibly take place in the way assumed by Garrey. Collections of this sort were observed in the case of all inorganic acids studied. They were not formed when distilled water alone was used. (3) The gatherings about bubbles of carbon dioxide of course could not occur in the manner described by Garrey, as the bubble contained no individuals to move out and form a ring.

In the case of the organic acids, the phenomena observed by us were precisely parallel to those occurring in inorganic acids. It is possible that the gatherings formed last longer in the case of some organic acids, though to us this seemed not usually very marked.

The results obtained by Garrey with organic acids varied much in different cases. With oxalic, formic, citric, succinic and valerianic acids the phenomena were the same as with inorganic acids; *i. e.*, a clear area was formed, sometimes with a ring of organisms surrounding it (if the acid was strong); this ring formation Garrey explained as in the case of inorganic acids.

" Malic, tartaric and mandelic acids produce a clear area, often with a ring about it. In the formation of the ring, the phenomena were so inconstant that I was unable to say that it was or was not due to a migration of the organisms from without to it " (*loc. cit.*, p. 307).

Finally, with acetic, butyric and lactic acids, a clear area surrounded by a dense ring was formed, and Garrey was able to assure himself that the ring formation was due to a migration of the organisms from the outside.

Thus Garrey's results with acids can be placed in three categories. (1) Some showed a ring formation, which in the author's opinion was due merely to the driving of the organisms out of the area in which the acid was found. (2) Some gave such inconstant results that the author was not certain what he should conclude about them. (3) Some showed a ring formation of such density and clearness that it was evident that the organisms came from outside of the acid area.

Now this inconstancy and uncertainty in the results with acids is exactly what is obtained when the carbon dioxide is not removed from the water by thorough aeration before each experiment. The culture water contains varying amounts of carbon dioxide and in some cases a part of it is accidentally driven off in the manipulations preparatory to the experiments, in other cases not. The presence of carbon dioxide means also the presence in the water of whatever it is that gives acids their characteristic qualities. Hence in such a fluid the organisms are already in an acid solution and naturally do not react with any precision when an acid is introduced, while in cases where the carbon dioxide is partly or entirely driven off, distinct reactions are obtained; the results thus become inconstant and uncertain. After careful removal of the carbon dioxide before every experiment, the results with all acids are, according to our observations, essentially the same, — *i. e.*, a ring or group is formed by immigration of the organisms from the outside. It was this same neglect to remove the carbon dioxide from the water that led Garrey to deny the results with Paramecium, though these are demonstrable with ease.

In view of the contrasted results obtained by Garrey on the one hand and by ourselves on the other, it is much to be desired that some third person should reinvestigate the reactions of Chilomonas, testing the various methods used, observing the precautions set forth, and perhaps taking counsel by correspondence or conversation with both sides, that there may be no omission which might seem to vitiate the work. It remains of course possible that Chilomonas from different cultures reacts differently, though we have used dozens of different cultures and have observed no such difference.

Our own results on Chilomonas may now be summarized. This organism reacts to carbonic and other acids just as Paramecium does, forming dense collections in localized areas, where carbon dioxide is present. The spontaneous aggregations sometimes formed by Chilomonas may therefore be due to their collection in carbon dioxide excreted by themselves.

Cyclidium glaucoma. — This ciliate infusorian likewise gathers in carbon dioxide and in solutions of acids in general. The collections thus formed are dense and lasting. Cyclidium was not observed to form spontaneous collections, though this may occur.

Colpidium colpoda. — This is one of the infusoria which was described by Pfeffer as collecting spontaneously into groups. It reacts to solutions of carbon dioxide and other acid solutions, just as Paramecium and Chilomonas do, gathering in dense aggregations about a bubble of CO_2, or in a drop of weak acid. It is therefore probable that the spontaneous groups are due to carbon dioxide. If Paramecium and Colpidium are mounted together, they will gather spontaneously into groups, each group containing both kinds of infusoria, the boundary of the groups being practically the same for each. The cause of the grouping is thus evidently the same in the two cases.

B. ORGANISMS WHICH FORM SPONTANEOUS GATHERINGS, BUT DO NOT COLLECT IN SOLUTIONS OF CARBONIC OR OTHER ACIDS.

Oxytricha aeruginosa. — This organism, when mounted on a slide, forms spontaneous groups which are similar in every respect to those formed by Paramecium. The method of reaction to a stimulus in Oxytricha is by backing, and turning to the aboral (or right) side, — the side which is not notched. If the organisms are at first scattered uniformly throughout the preparation, they will soon be found to be forming groups in one or more regions. If the individuals within the groups are observed, they are found to be swimming hither and thither in all directions. But when one comes to the outer boundary of the group, it at once swims backward a short distance, turns toward the aboral side, and then starts forward again. As this happens every time the boundary of the group is reached, the animal remains within it. Individuals outside, whose course carries them by chance into the areas where a group is forming, do not react at all as they enter the area. But after swimming across, they do react as above described upon coming to the outer boundary of the area. Hence every Oxytricha that enters a group remains within it, and after a time a dense aggregation is formed. The groups thus produced increase in area, spreading out regularly, but maintaining a definite boundary.

The phenomena seem thus in every way identical with those observed in the case of Paramecium (see the first and second of these studies). It might therefore be reasonably expected that the cause would be found to be the same. But experiment shows that this is not the case; Oxytricha aeruginosa does not collect in regions where carbon dioxide is present, nor in other acid solutions. If a bubble of carbon dioxide is introduced into the preparation, the Oxytrichas do not gather about it, but on the contrary give their " motor reaction " when they come into its neighborhood, — reversing the direction of movement, and turning toward the aboral side. They thus leave the space about the carbon dioxide empty. Toward drops of acid solutions of all sorts they react in the same manner.

If Oxytricha and Paramecium are present in the same culture, or if the two are mixed together and experimented upon in the usual way, the results are as follows. The Paramecia collect about the bubble of carbon dioxide, or in the drop of acid, at once; the Oxytrichas do not. Thus a separation of the two kinds of infusoria is soon brought about.

If Oxytricha and Paramecium are mounted together and the slide is allowed to stand for a time, both kinds of infusoria will form spontaneous groups, but the two groups are quite separate. The Paramecia gather in one region, the Oxytrichas in another. Individuals of either kind may pass directly across the groups formed by the other, or swim in and out of the area where the other group occurs. The groups are thus clearly due to different causes in the two cases.

Oxytricha therefore forms spontaneous gatherings similar to those of Paramecium, but not due to the same cause. It seems evident that Oxytricha must excrete some other substance, not an acid, which acts upon it in the same way that the excreted carbon dioxide acts upon Paramecium. The nature of this substance remains to be discovered.

Loxocephalus granulosus. — In the case of this organism the facts are closely parallel to those described for Oxytricha aeruginosa. It forms spontaneous gatherings, but does not collect about bubbles of carbon dioxide nor in acid solutions in general. Mounted on the same slide with Paramecium, the two organisms form separate groups in different regions of the preparation. Clearly, Loxocephalus, like Oxytricha, excretes some substance which brings about the collections, but this substance is not carbon dioxide.

In preparations containing both Paramecium and Loxocephalus, the following may be observed as to the relations of the two organisms. Loxocephalus swims in and out of the groups of Paramecia, paying no attention to the limits so strictly observed by the Paramecia. In the same way Paramecium swims indifferently in and out of the groups of Loxocephali, when the latter groups are first forming. But after a group of Loxocephali has become well established and contains very large numbers of individuals, a Paramecium passing accidentally into the group usually remains there. Thus after a time a considerable number of Paramecia may be mingled with the Loxocephali. The Paramecia swim about freely within the group, but turn back on coming to an outer limit. It is to be noted that this outer limit is not the same as that which turns back the Loxocephali, but lies a little outside of it, so that the area in which the Paramecia are confined is larger than that which limits the Loxocephali, inclosing the latter.

These phenomena are probably to be explained as follows. Loxocephalus is not affected by carbon dioxide, therefore does not gather in the groups formed by the Paramecia, but swims in and out of them indifferently. But it does excrete some other substance, not of an acid nature, into which it gathers; hence the spontaneous collections formed. To this substance Paramecium is indifferent, hence it swims indifferently in and out of the groups of Loxocephali, at first. But of course Loxocephalus produces carbon dioxide in its respiratory processes, hence after a group of these organisms has been formed for some time, the water becomes impregnated with carbon dioxide, as well as with the other (hypothetical) substance. Paramecia now passing into the group remain, owing to the carbon dioxide. The areas over which the carbon dioxide and the hypothetical substance are effective are not identical, that for the carbon dioxide being a little larger; hence the limit of the excursions of the Paramecia is outside that for the Loxocephali.

C. ORGANISMS WHICH DO NOT COLLECT IN CARBONIC OR OTHER ACIDS, AND WHICH WERE NOT OBSERVED TO FORM SPONTANEOUS GATHERINGS.

The following organisms were tested with carbon dioxide and with solutions of various acids in the same manner as those hitherto described. Every precaution was taken to remove the carbon dioxide from the water before making the tests, and the experiments were repeated under various conditions, with uniform results. None of these organisms gather about bubbles of carbon dioxide or in solutions of acids. They are as follows: Oxytricha fallax, Euplotes charon, Stylonychia pustulata, Colpoda cucullus, Spirostomum teres, Stentor cæruleus, Enchelys farcimen, Halteria grandinella, Didinium nasutum, Euglena viridis, and Heteromita globosa.

Some of these organisms, on coming in contact with a solution of carbon dioxide, at once give their characteristic "motor reaction," backing and turning toward a structurally defined side; thus they turn away from the area in question, leaving it empty. Others do not react at all to carbon dioxide, and to other acids only when very strong. Those that were indifferent were Oxytricha fallax, Stentor cæruleus, Didinium nasutum, Euglena viridis, and Heteromita globosa.

D. THE METHOD BY WHICH THE GATHERINGS ARE BROUGHT ABOUT.

Throughout the work attention was given to the method by which the infusoria gather together. The point which was especially studied was the question of orientation. Do the organisms collect in the region where a certain chemical is present because they become oriented in the lines of the diffusing ions? Or are the collections brought about in the manner described for Paramecium, in the first and second of these studies?

The phenomena were carefully examined in all the infusoria in which collections were observed, — in Colpidium colpoda, Oxytricha aeruginosa, and Loxocephalus granulosus, and additional observations were made on Paramecium and Chilomonas. All the ciliates mentioned are of sufficient size so that their movements can be exactly observed with the Braus-Drüner stereoscopic binocular, and there can be no doubt as to the method in which the gatherings take place. They collect in essentially the same manner as has been shown in previous studies to be true for Paramecium. The organisms are at first swimming freely hither and thither. When the drop of acid is introduced, or collections are produced in other ways in certain regions, some of the individuals swim into the area in question merely through their usual movements. They do not change their course or react at all as they enter the area. But as they swim across it and reach the opposite side, where they would if unchecked pass out of the area into the surrounding water, each infusorian gives its characteristic " motor reaction." Oxytricha after moving backward turns toward its unnotched side, Loxocephalus to the aboral side, Colpidium toward its convex side, Paramecium toward the aboral side. The animal is thus prevented from leaving the area containing the chemical, but swims in another direction within this area. As it reacts in the same way every time it comes to the outer boundary of the area, it does not leave it at all. Other individuals enter in the same way, through their random movements, and remain through the same reactions, so that after a time the areas in question swarm with infusoria.

If the animals are allowed to come thoroughly to rest before introducing the chemical, usually no collection is formed within it. This shows the essential part played in the reaction by the random movements of the organisms.

It seems difficult for many minds to believe that the dense gatherings observed can be produced in this way. That this is the real method by which the collections occur, can be very neatly demonstrated to the eye in the following manner. A number of Paramecia or other infusoria which collect in acids are mounted on a slide. Upon the upper surface of the cover glass a small circle about the size of the drop of acid usually introduced, is made in ink with the pen. By directing the attention to the area within the ring of ink, it will be seen that many infusoria (as many as ten per second or more, in an ordinary mount of Paramecium), cross the area every instant. It is therefore evident that if all of them could be stopped within the area, a dense group would soon be produced. With the capillary pipette a drop of acid is now introduced beneath the ring; the same number of infusoria now enter the area as before, but every one remains and a dense collection soon results.[1]

In the paper already cited, Garrey maintains that the flagellate Chilomonas collects in certain acids in a manner entirely different from that above set forth. He holds that the collections (in acetic acid, for example), are produced through an orientation of the organism in the lines of the diffusing ions. The reactions to other substances, drops of which are left empty by Chilomonas (as for example a solution of sodium chloride), take place in a way entirely different, according to Garrey. Here there is no orientation; the chemicals merely cause "swift shooting movements," by which the animal is carried out of the area, or prevented from entering it. The method of reaction exhibited in collecting in acetic acid is denominated by Garrey *chemotaxis*, while that shown in keeping out of or leaving a drop of sodium chloride he calls *chemokinesis*.

In the sixth of this series of studies, reasons drawn from a study of the movements of the individual Chilomonads have been given for rejecting this distinction in kind between the reaction in collecting and that in avoiding a region containing chemicals. Certainly no such distinction can be made in Paramecium, nor in the other ciliates above mentioned. Leaving out of account the direct observations on the movements of the individuals, there are certain experiments which amount almost to a demonstration that there is no such distinction in kind, — even in Chilomonas. They demonstrate at least that collections exactly similar to those produced through the supposed "chemotaxis" can be produced through the operation of the admitted "chemokinesis."

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[1] This experiment was demonstrated on the screen by means of the stereopticon before the Society of Western Naturalists at the meeting in Chicago in December, 1900.

Acetic acid may be taken as a type of the substances toward which, according to Garrey, Chilomonas shows orientation, or "chemotaxis;" while sodium chloride is an example of the substances which cause no orientation, but merely "chemokinesis." If a drop of acetic acid of a proper concentration is introduced into a preparation of the infusoria, the latter soon collect in the drop ("chemotaxis"), while if a drop of sodium chloride solution is introduced, it remains empty ("chemokinesis").

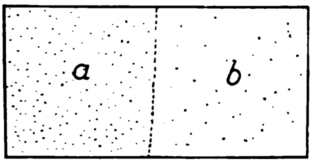

FIGURE 4.

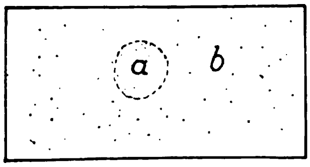

FIGURE 5.

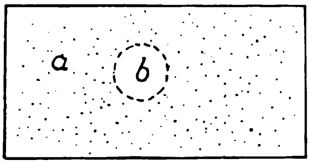

FIGURE 6.

FIGURES 4, 5, and 6. Diagrams showing how the grouping of the organisms depends on the relations of the two fluids to each other in space. *a* represents the area occupied by the fluid into which the infusoria may pass without giving the "motor reaction;" *b* the fluid into which it cannot pass without giving the "motor reaction." When *a* is water, *b* is a salt or alkaline solution; when *b* is water, *a* is an acid solution; in either case the grouping of the organisms (Paramecium or Chilomonas), is that shown in the figures.

But suppose we mount our infusoria in a weak solution of sodium chloride, and introduce a drop of water? The salt solution is admitted to produce no orientation, but merely "chemokinesis," yet in a short time the drop of water is filled with a dense group of infusoria,—just as was the acetic acid in the former case. Apparently the collection is formed in water just as quickly as in the acid, and the present authors have been able to detect no difference in the method of formation. It is at least demonstrated that collections can as well be formed without orientation as with it, and that if these infusoria possess the power of becoming oriented to diffusing ions, this power is a useless luxury.

According to our observations, the phenomena are identical in the two cases. The organisms swim about, in the solution in which they are mounted (water, or sodium chloride solution, respectively), and enter the drop (acetic acid, or water, respectively), without reaction. After having entered they give the usual "motor reaction" when they come to the outer boundary of the drop; hence they do not leave it, and the drop after a time swarms with the animals.

The following series of experiments is instructive and brings out clearly the facts as they appear to the present authors.

Mix a part of the infusoria (Paramecium or Chilomonas) with a weak solution of sodium chloride, not strong enough to injure them, mix others with a weak, non-injurious solution of acetic acid, and leave others in water. Now make mounts with the fluids in various relations to each other:

(1). Make a preparation (Fig. 4) in such a way that half the fluid on the slide is water (*a*) containing infusoria, while the other half is salt solution (*b*) containing infusoria. After a short time most of the infusoria will be in the half containing water alone.

(1a). Make a similar preparation (Fig. 4), save that one half (*a*) is acetic acid containing infusoria, the other half (*b*) water containing infusoria. In this case after a time most of the organisms will be found in the acid.

(2). Make a preparation (Fig. 5) in such a way that the salt solution surrounds the drop of water, the water (*a*) being introduced as a drop into the salt solution (*b*). After a time the drop (*a*) of water contains a dense swarm of the organisms.

(2a). Make a preparation as in the last, save that water (Fig. 5, *b*) surrounds the acid (*a*), which is introduced as a drop into the water. In this case there is likewise a dense aggregation formed in the drop *a* (of acid).

(3). Make a preparation (Fig. 6) such that the water (*a*) surrounds the salt solution (*b*),— the latter being introduced as a drop into the water. After a short time the drop *b* (of salt solution) is empty.

(3a). Make a similar preparation, in which the acid solution (Fig. 6, *a*) surrounds the drop of water (*b*). Soon the drop *b* (of water) is left empty.

With the same pair of substances we get, therefore, either a dense aggregation (or what has been sometimes called "positive chemotaxis"), or a certain definite area left vacant (" negative chemotaxis"), depending upon the relation in space of the two fluids to each other. And this result may be obtained whether we use as our chemical one like acetic acid, to which it has been maintained that the infusoria show positive "chemotaxis" proper, or whether we employ a salt to which they are held to react only by "chemokinesis."

Thus with either pair of fluids, whether we do or do not get a dense aggregation of infusoria depends "on the configuration of the two fluids" — on the relation of the two fluids to each other in space. General statements embodying these relations may be made as follows. If we distinguish as *b* that fluid into which the infusorian cannot pass without causing the "motor reaction," as *a* that into which it can pass without causing the reaction, then

If *b* surrounds *a* (Fig. 5), a dense aggregation is formed in *a* ("positive chemotaxis").

If *a* surrounds *b* (Fig. 6), the small area *b* is left empty ("negative chemotaxis").

If *a* and *b* occupy equal areas (Fig. 4), after a time most of the organisms will be found in *a*. (This last case is not so strongly realized in a minute organism like Chilomonas as in a larger creature, such as Paramecium, because the distances to be passed over are so great that a weak swimmer like Chilomonas will not soon reach the area *a*, and may come to rest in large numbers in *b* without reaching *a* at all. But in any case, a considerable majority will be found in *a*.)

If *a* is water, *b* may be a solution of an alkali or of a great variety of neutral salts; in the case of Paramecium, almost any neutral salt If *b* is water, *a* may be any acid. In either case the resulting phenomena will be essentially the same.

SUMMARY.

1. In order to test the reactions of infusoria to acids, it is necessary to remove with great care from the water containing the organisms the carbon dioxide produced by the organisms in their respiratory processes.

2. Colpidium colpoda, Cyclidium glaucoma, and Chilomonas paramecium collect in solutions of carbonic and other acids, just as Paramecium does. The spontaneous collections formed by these organisms may therefore be due to their excretion of carbon dioxide.

3. Loxocephalus granulosus and Oxytricha aeruginosa form spontaneous collections similar to those of Paramecium, but do not gather in carbonic or other acids. The spontaneous collections in these cases must therefore be due to other causes.

4. The following infusoria do not collect in carbonic or other acids,

nor were they observed to form spontaneous gatherings: Oxytricha fallax, Euplotes charon, Stylonychia pustulata, Colpoda cucullus, Spirostomum teres, Stentor cæruleus, Enchelys farcimen, Halteria grandinella, Didinium nasutum, Euglena viridis, Heteromita globosa.

5. The collections, according to our observations, take place in the manner described in previous numbers of this series of studies for Paramecium. In cases where this has been disputed, it is shown that collections essentially similar to those produced by what has been considered " chemotaxis " proper are likewise produced by what is admittedly " chemokinesis."

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STUDIES ON REACTIONS TO STIMULI IN UNICELLAR OR GANISMS. IX. — ON THE BEHAVIOR OF FIXED INFU SORIA (STENTOR AND VORTICELLA), WITH SPECIA REFERENCE TO THE MODIFIABILITY OF PROTOZOA REACTIONS.

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STUDIES ON REACTIONS TO STIMULI IN UNICELLULAR ORGANISMS. IX. — ON THE BEHAVIOR OF FIXED INFUSORIA (STENTOR AND VORTICELLA), WITH SPECIAL REFERENCE TO THE MODIFIABILITY OF PROTOZOAN REACTIONS.[1]

BY H. S. JENNINGS.

THE only infusorian whose behavior is at present known with any degree of fulness is Paramecium. This animal is a type of the free-swimming infusoria; while Paramecium does at times come to rest, as a rule it is found in rapid movement, — especially when under experimental conditions.

The behavior of an animal which is fixed in a definite position will necessarily be of a different character from that shown by such an organism as Paramecium. Stentor and Vorticella furnish examples of such animals, and, as will appear, their behavior differs much from that of Paramecium, — showing indeed a much higher development.

As compared with an organism continually in motion, a fixed animal offers many advantages for the experimental study of behavior, for one may keep the same individual continuously under observation, or return to it at longer or shorter intervals. It is thus possible to observe changes in behavior, and to determine whether the reaction to a given stimulus is modified by previous subjection to the same or different stimuli.

The present paper is based upon a study of the behavior of the following organisms: Stentor rœselii Ehr., Stentor cæruleus Ehr. several species of Vorticella, Epistylis flavicans, var. procumbens, and Carchesium polypinum Lin. Stentor is much more favorable for such work than any of the Vorticellidæ, and Stentor rœselii is in many respects the most favorable as well as the most interesting of the organisms studied. I shall therefore make an account of the behavior of this animal the basis of the paper, comparing the others with it.

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[1] Contributions from the Zoölogical Laboratory of the University of Michigan, No. 57.

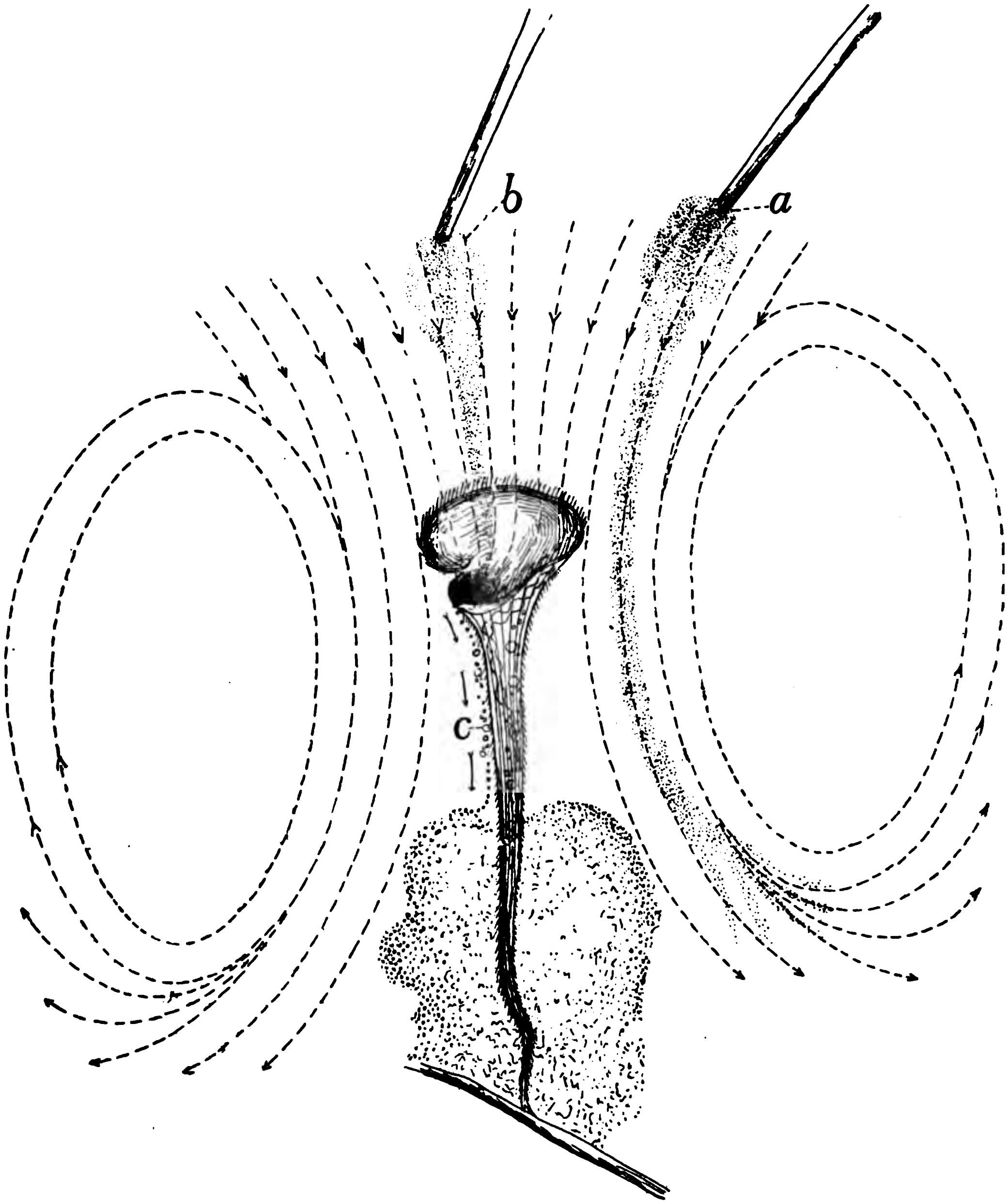

Figure 1. — Stentor rœselii, showing the currents caused by the cilia. At *a* a chemical is introduced a little to one side of the disc, showing that it does not reach the animal. At *b* a chemical is introduced above the disc; it is carried directly to the mouth. At *c* particles are seen passing along the ventral surface of the body to the edge of the tube.

A. THE BEHAVIOR OF STENTOR RŒSELII EHR.

For understanding the behavior of an organism when subjected to stimuli, it is necessary to have well in mind the structure of the animal and its normal movements when unstimulated, — a consideration too often neglected in work on behavior.

Stentor rœselii Ehr. (Fig. 1) is a colorless or whitish animal consisting, when fully extended, of a slender, tapering, stalk-like body, bearing at its larger end a broadly expanded disc. The surface of the body is covered with longitudinal rows of fine cilia, and bears also a considerable number of fine long setæ, which disappear at times, and are said to be retractile and extensile. The disc is surrounded by a circlet of large compound cilia or membranellæ. These make a spiral turn, passing on the left side into the large buccal pouch, which leads to the mouth. The mouth thus lies nearly in the middle of what may be called the oral surface; this surface is considered ventral in determining right and left. The disc is covered with rows of fine cilia which are nearly parallel with the circle of membranellæ. The smaller end of the tapering body is known as the foot; here the internal protoplasm is exposed, sending out fine pseudopodia, by which the animal attaches itself to objects (Fig. 2).

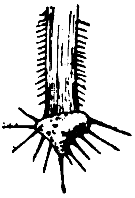
FIG. 2. — Foot of Stentor rœselii, after Johnson ('93), showing the pseudopodia.

The body contains, next to the surface, many fine contractile fibrillæ, the myonemes, through the action of which the animal may contract into a short oblong or conical form (Fig. 3).

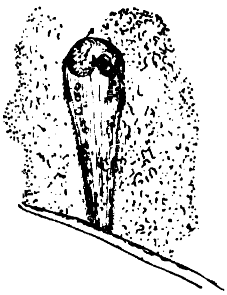
FIG. 3. — Stentor rœselii, contracted into its tube.

Stentor rœselii is usually attached to a water plant or a bit of débris by the foot, and the lower half of the body is surrounded by the so-called tube. This is a very irregular sheath, formed of flocculent material of all sorts, partly held together by a secretion from the Stentor. It is frequently nearly transparent, so as to be almost invisible. The manner in which the tube is formed will be described later.

Movements of cilia in the unstimulated animal.[1] — The membranellæ and cilia of the oral disc are in continual motion in the extended animal; the nature of this motion is best seen by adding something to the water to make the currents induced by the cilia visible. When finely ground sepia or carmine is added to the water, the currents caused by the cilia are seen to be as follows: The mouth of the animal forms the bottom of a vortex, towards which the water above the disc descends from all sides (Fig. 1). Only the particles in the water near the axis of the vortex really strike the disc, — those a little to one side shoot by the edges without touching. These latter curve outward again after reaching a point below the disc, and thus a whirlpool is produced, — some of the particles returning upward so as to reach again the downward current at a point some distance from the Stentor. But most of the particles which thus miss the edge pass downward and out of the sphere influenced by the Stentor.

Particles which reach the disc pass to the left, toward the buccal pouch, showing that the beat of the membranellæ has a component which drives to the left as well as downward, — the real movement of the current being thus a left spiral. The particles thus reaching the buccal pouch are whirled about within it a few times, then they may take one of two courses. They either pass down into the mouth at the bottom of the pouch and thus into the internal protoplasm, or they are whirled out over the edge of the pouch, in the mid-ventral notch. In the latter case they usually pass backward toward the foot of the animal, along the mid-ventral line, as shown at *c* in Fig 1. Apparently the body cilia in this region keep up a backward current. The particles reach the edge of the tube, where they may cling, thus aiding to build up the tube.

In determining whether certain given particles shall pass into the protoplasm or out over the edge of the disc, there seems to be no indication of sorting by the cilia and of choice, — though this would not be at all surprising in view of what we know of choice in Amœba, and of corresponding phenomena in inorganic fluids (see Rhumbler, 1898, or the brief resumé in Jennings, 1902). But in Stentor, as long as the disc remains extended, whenever particles of any sort are allowed to reach the disc in large numbers, some are taken into the protoplasm, while others pass over the edge and away, without regard to the nature of the particles (provided they are not too large; see p. 30). This is true, for example, of sepia, carmine grains, unicellular algæ and débris of all sorts. When large numbers of minute unicellular algæ pass into the buccal pouch, apparently the proportion taken into the protoplasm is the same as in the case of sepia or other non-nutritious particles. It seems evident that whether a given particle shall or shall not be taken into the internal protoplasm depends upon the mechanical conditions governing the spiral currents in the pouch. Many of the particles in the current never reach the minute mouth at all, and these are whirled over the edge in continuation of their spiral course; those which are so situated in the vortex as to be carried directly to the mouth are taken in. Of course Stentor does exercise a sort of choice (as will appear below), by changing its position, reversing the ciliary current, or contracting when injurious substances are present in the water, but there is no indication of a sorting and selection of particles brought into the pouch by the usual currents.

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[1] It is doubtless to be held that the animal is never really unstimulated; the use of this term signifies merely that no special stimulus is acting on the animal, beyond what is supplied by the usual conditions of existence.

When stimulated, Stentor rœselii may contract into its tube (Fig. 3). Such contractions do not as a rule take place except in response to well marked stimuli. This was the rule throughout my observations, extending over many days. Undisturbed individuals observed without interruption for an hour or more did not contract at all during that time. In this respect Stentor differs from Vorticella, which contracts at short intervals, even when the conditions are apparently quite uniform (see Hodge and Aikins, 1895).

Reactions to stimuli. I. Mechanical stimuli. — We will first consider what Stentor does when touched or struck by small objects, — its reactions to simple mechanical stimuli. Such stimuli are often received in the normal life of Stentor, and there is a surprisingly full and complicated set of reactions to them, as compared with the simple reactions of Parmecium.

We will suppose that small solid bodies are brought with the water currents to the disc. This may be controlled experimentally by drawing a glass tube to a long, excessively fine capillary point, filling the tube with water containing finely ground sepia, and bring the point near the Stentor. What the animal does depends upon a number of different conditions.

Normal movements continued. — At first the normal currents are not changed; the particles pass into the pouch, and some are taken into the internal protoplasm, while others pass out over the edge of the pouch at the mid-ventral notch, as described above. If the particles are minute, do not cling together into large masses, are not excessive in number, nor mingled with any stimulating chemical, the currents continue this normal course indefinitely.

Bending toward the source of stimulus. — If a small object merely touches gently one edge of the disc, the Stentor may bend over toward the object (Fig. 4). This reaction may be seen when a small organism comes against the disc of Stentor, then attempts to swim away. The Stentor bends in that direction, so as to keep in contact with the object as long as possible. In the culture dishes containing Stentors there were many free heads of Epistylis, and these frequently swam thus against the Stentors, giving rise to the above described reaction. The Epistylis heads were not held at all, but were merely followed as far as possible by bending.

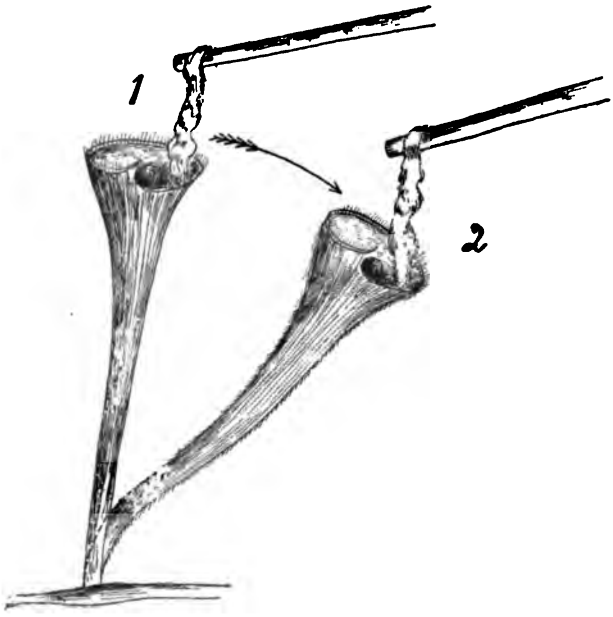

FIGURE 4. — Stentor rœselii bending in the direction of a slight mechanical stimulus. At 1 a bit of débris is allowed to touch the edge of the disc, and is then pulled to the right. The Stentor follows it, bending into the position shown at 2.

This reaction may be produced experimentally by tickling the edge of the disc with the tip of a minute glass rod drawn out to the finest possible hair.[1] The Stentor bends over toward the side touched, and if the rod is moved very gently to one side, follows it.

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[1] These and similar manipulations were carried out under the Braus-Drüner binocular microscope, the use of which renders very simple many experiments and observations which would otherwise be difficult.

If the rod trembles a little too much, the Stentor will contract suddenly, as described below. The most satisfactory way of producing the reaction is to get a bit of soft flocculent débris from the bottom of the dish to cling to the rod. This débris may then be allowed to come against the disc, and is then gently pulled to one side. The Stentor follows it, often bending far over. This experiment is represented in Fig. 4. The animal may thus bend in any direction, — to the right, to the left, or toward the oral or aboral side.

Bending away. — If the stimulus is a little stronger, as one produced by a large hard object, or by the objects becoming too numerous, as when a dense cloud of sepia reaches the disc, or when the objects are accompanied by a weak chemical stimulus, as is the case with carmine grains, then another reaction is produced. The animal bends away from its present position. This is thus to a certain degree the opposite of the reaction last described, but is not so precisely localized a reaction as the former one. In this reaction the organism shows the influence of its spiral, unsymmetrical structure, in that, as in the case of Paramecium, it always turns toward a structurally defined side. The reaction in Stentor is as follows: the animal twists on its long axis one or two turns, then bends over toward the aboral side. It thus bends into a new position, but it does not always bend *away* from the source of stimulus; in some cases this reaction carries the animal toward the source. In the latter case the reaction is repeated.

In most cases where the source of stimulus is not large, this reaction succeeds in removing the Stentor from its action. Thus, if a capillary tube containing sepia is held close to the disc, when the animal bends over toward the aboral side the particles of sepia no longer reach the disc, and the animal is relieved from the stimulus (Fig. 5). Much experimentation shows that this simple reaction is more effective in getting rid of stimuli of all sorts than might be anticipated. If the first reaction is not successful in accomplishing this end, it is repeated.

Reversal of the ciliary current. — If the turning toward one side does not relieve the animal (or in some cases before this is tried), so that the particles continue to come in a dense cloud, the ciliary current is suddenly stopped and apparently reversed for an instant. The particles in the pouch or against the disc are thus thrown off. The reversal lasts but an instant, then the current is continued. If the particles still continue to come, the reversal is repeated two or three times in rapid succession. If this fails to relieve the animal of the stimulus, the next reaction (contraction) usually supervenes.

Sometimes this reversal of the current takes place before the turning away described above, and it may be followed by that reaction. But usually the turning away occurs first.

The reversal is produced under various circumstances. It occurs when a very large number of particles reach the disc at once, so that there is a tendency to clog the pouch, or when a large hard object, such as one of the loricate Ciliata, gets into the pouch. I have seen Coleps gotten rid of in this way. It occurs also when some chemical, as a weak salt solution, is mingled with the particles, or when the chemical alone reaches the disc (see the reactions to chemical stimuli, below).

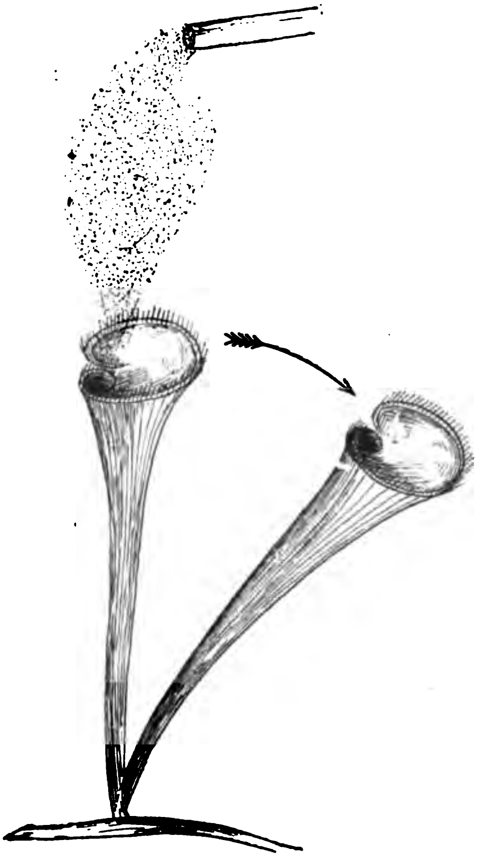

FIGURE 5. — Stentor rœselii bending away when a quantity of sepia or of some chemical reaches the disc. The animal bends toward the aboral side.

Contraction. — If the animal does not succeed in getting rid of the stimulus in either of the ways above described, or if the stimulus is a very powerful one to begin with, the Stentor suddenly contracts. The body becomes short and club-shaped or oblong, and the Stentor disappears within its tube (Fig. 3). Here it usually remains twenty to thirty seconds, then rather slowly extends, so that from the moment of contraction to the moment of complete extension an interval of 40 to 50 seconds has usually elapsed.

When, in extending, the body of the Stentor has become about half or two-thirds its original length, the ciliary disc begins to unfold and the cilia to act, causing the current to reach the disc as before. If with the current the stimulus again acts upon the animal (as when the sepia or the chemical is kept near), immediate recontraction follows.

This may be repeated many times. To certain sorts of stimuli, as will be seen later, Stentor may get accustomed, so as to unfold and behave in the usual manner while the stimulus continues undiminished. We will consider for the present the case where the continuance of the stimulus involves continued repetition of the reaction. This case is realized when a dense cloud of carmine grains is kept where it will strike the Stentor as soon as it expands, or when various chemicals are kept in this position. In such a case the contractions are repeated, as above described, usually for a period of ten to fifteen minutes. Often the animal, after a number of contractions, remains within its tube a longer time than at first. But more often there is little change in the time of contraction until toward the end of the period of ten or fifteen minutes. If the stimulus continues, the next phase of the reaction now sets in, described in the following.

Abandonment of the tube. — After the stimulus has been thus repeated at every unfolding of the Stentor for ten to fifteen minutes, the animal contracts violently several times, without intervening full extension. The short clavate body merely lengthens a little, then contracts suddenly and powerfully into a still shorter mass. This is repeated until the attachment of the foot of the Stentor at the bottom of the tube is broken, and the animal is free. It now leaves the tube and swims away. The animal may swim forward out of the anterior opening of the tube, but if this takes it into the sphere of operation of the stimulus, as will very often be the case, it may force its way backward through the substance of the tube, and thus gain the outside, swimming backward. It then swims away, to form a new tube elsewhere.

Behavior while free. — While thus swimming through the water, after leaving its tube, Stentor takes on the characteristic behavior of the free-swimming infusoria, such as Paramecium. In the open water stimuli are almost lacking for the guidance of the animal, hence its behavior is, paradoxical as this may seem, much less free and varied than is that of the fixed infusorian, or the infusorian creeping on the bottom; it becomes quite stereotyped. The writer has previously given (Jennings, 1899*b*) an account of the main features in the behavior of Stentor polymorphus when swimming in the open water. The behavior of Stentor rœselii is essentially similar in character. It rotates to the left on its long axis as it swims, and at the same time it swerves toward one side, — apparently toward the right aboral side. Its path thus becomes a spiral, like that of Paramecium (for the significance of this spiral swimming, see Jennings, 1901). When the Stentor in its course comes into the region of a stimulating chemical or other stimulating agent, the animal swims backward a little, turns toward the right aboral side, and swims forward again. In all these respects its behavior is essentially like that of Paramecium, as described in the second of these studies (Jennings, 1899*a*), so that it will not be described in detail here. At first after leaving the tube the Stentor is strongly contracted, of a very short oblong or club-shaped form. Usually as it swims it gradually extends a little, taking a long conical form, but remaining much shorter than the fixed specimen. The animal thus swims rapidly for some time about the vessel in which it is confined. It may be observed that the Stentor as it swims secretes over the posterior half of its body a transparent mucus or sticky substance of some sort, since carmine grains or other small particles in the water often cling to the posterior half of the body, or are trailed along some distance behind it, — the mucus evidently pulling out to form threads.

On coming against the surface film or the smooth surface of the glass, the Stentor behaves in a peculiar way. The (only partly unfolded) disc is applied to the surface, and the animal creeps or spins rapidly over the surface, often revolving to the left; sometimes not revolving, and always progressing in the direction of the right aboral side or angle of the disc.

On coming in contact with a bit of plant tissue or débris (consisting in the cases observed largely of worm-castings), the Stentor usually creeps rapidly over the débris, keeping the ventral surface against it. It thus follows all the irregularities of the surface, as rapidly and neatly as this would be done by one of the Hypotricha. This may continue for some time, the animal seeming to explore the object thoroughly; then it may leave the débris and swim about freely again for a period. At times the Stentor becomes attached to a piece of débris by the secreted mucus. This is drawn out to form a thread, often several times the length of the Stentor; by means of this thread the Stentor remains suspended in the water, as it were, whirling about on its long axis. It may thus remain partially attached for some time; then the thread is broken by a sharp contraction of the body, and the animal swims away.

Formation of a new tube, and attachment of the foot. — Finally (in three cases that were timed, after fifteen to twenty minutes) the Stentor forms a new tube and attaches itself. This is done as follows. The animal, coming to a small heap of débris, creeps over it with ventral surface against it, as above described, exploring it thoroughly. It becomes evident that mucus is being secreted over the surface of the posterior half of the body, since particles of débris stick to the body, or are trailed behind it. Finally, in a certain region, often between two masses of débris, the animal begins to move backward and forward, through a distance of only about three fourths of its own length (when contracted). This is kept up for about two minutes, and results in the formation of a short mucus sheath, from the secretion on the outer surface of the Stentor. This process is illustrated in Fig. 6. Now the foot is pressed against the débris at the posterior end of the sheath, where it adheres, — doubtless by the extrusion of pseudopodia, as illustrated in Fig. 2. Now the Stentor extends its body to the full length, — and we find it in the usual attached condition, with the lower half of the body surrounded by a transparent tube of mucus.

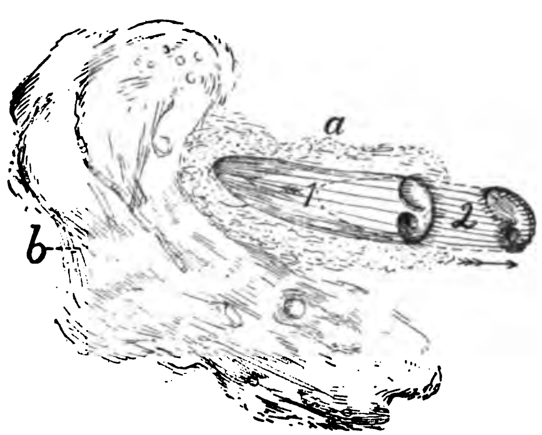

FIGURE 6. — Illustrating the movement of Stentor in forming a new tube. The animal oscillates between the positions 1 and 2, giving off mucus, which forms the tube. *a*, mucus forming the tube; *b*, débris.

The above account is drawn from observation of the process of settling down and forming a tube in several specimens, and seems to be typical. In one case observed, however, the animal attached itself to the smooth surface of the glass, and this time the process differed. After wandering about for some time, as described above, the specimen applied its disc to the bottom of the vessel, and revolved for some time on its long axis. Then it ceased revolving, and slowly bent its body till the foot reached the bottom, — the body becoming nearly straight again and tangential to the surface, before this was accomplished (Fig. 7). The foot attached itself to the bottom, then the disc was lifted up, and the body took a position perpendicular to the surface. The animal was now attached in the usual way, though the beginning of the tube had not been made. The tube in such a case is formed later, automatically, as it were, by the secretion of mucus on the surface of the body. This becomes compacted and confined to the posterior half of the body by the contractions of the Stentor in responding to stimuli.

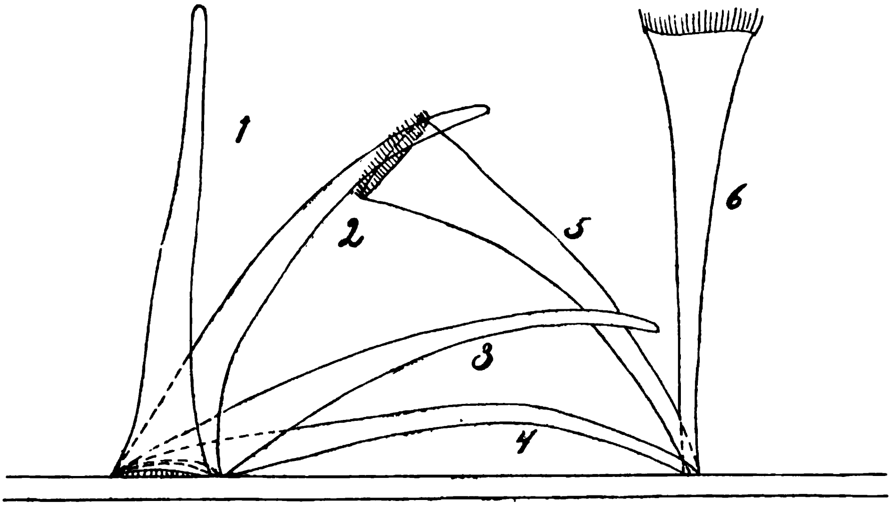

FIGURE 7. — Illustrating the manner in which Stentor rœselii attaches itself to a smooth surface. The figures 1–6 represent the successive positions occupied by the Stentor.

Usually, however, the tube is formed before the attachment of the foot, in the manner first described.

Having thus described the typical series of reactions when Stentor rœselii is subjected to mechanical stimuli, or to a combination of mechanical and chemical stimuli, we may return and consider the effect of some other stimuli, as well as a number of matters of a different character.

II. Chemical stimuli. — Results essentially the same as those above described are obtained in stimulating Stentor rœselii by means of chemicals. But there are certain points which are of much importance for understanding the method by which such organisms react to chemicals; these will be brought out here.

When a chemical of sufficient strength to act as a stimulus, yet not strong enough to be destructive, is allowed to reach the disc of an attached Stentor, the same series of reactions is given as has been described above, — changing position, reversal of ciliary current, contraction, and final abandonment of the tube. These results were obtained with a weak solution of methyline blue; with the red filtrate from carmine in water, with $\frac{m}{100}$ NaCl, with $\frac{m}{100}$ HCl, and with $\frac{m}{10}$ cane-sugar. In the latter case the effect was evidently due to the osmotic action of the sugar, as will be shown later. Other chemicals were not tried.

After it was found that Stentor would bend directly toward the source of a weak mechanical stimulus, as described above, it was thought possible that an opportunity might be here presented for demonstration of positive or negative chemotropism, — a bending to or from the source of diffusion of a chemical. In other infusoria the writer has been unable to observe a direct turning toward or away from the source of diffusion of any chemical, so that this seemed an opportunity not to be missed. The experiments in this direction developed certain facts which are of much significance for understanding the reactions not only of Stentor, but of other ciliates and flagellates, to chemicals.

The attempt was made to localize very accurately the action of the stimulus, by the use of fine capillary tubes, bringing the chemical near to one side of the body, — so that it might affect one side alone. The Stentor might then be expected to bend toward or away from the side affected. This involves no difficulty in manipulation, but an insuperable difficulty is at once met in the course of the currents produced by the cilia of Stentor. Chemicals placed at one side do not reach the animal at all, as will be seen by an inspection of the course of the currents in Fig. 1. The chemical at *a* is carried past the animal without touching it. This is rendered evident when some colored chemical, such as a solution of methyline blue, is used. If the point of the tube is moved farther toward the front of the Stentor, the solution is involved in the central vortex and is carried directly to the buccal pouch and the mouth (as at *b*, Fig. 1).

Thus unilateral stimulation with a dissolved chemical, elsewhere than at the mouth, is practically impossible. This is true also when the chemical in solution is advancing with a broad, plane front, as illustrated in Fig. 8. In such a case the solution does not reach the Stentor uniformly distributed, as determined solely by the movements of the ions. On the contrary, as soon as the advancing solution has arrived within a certain distance of the animal, a small cone of the substance is drawn out by the vortex, directly toward the disc of the animal. The point of this cone reaches the buccal pouch and mouth of the Stentor, long before the rest of the chemical has affected the animal. This is very clearly seen when colored chemicals are used.

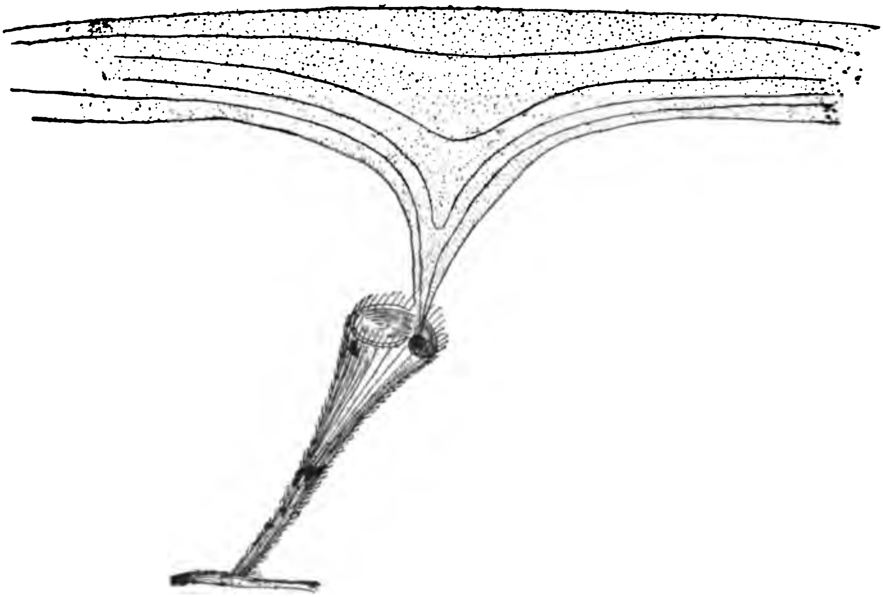

FIGURE 8. — Illustrating the way in which an advancing chemical is drawn out in the alimentary vortex, so as to reach the mouth and disc of Stentor without affecting the remainder of the body.

The result is that the animal always receives its stimulus from a chemical at a certain definite spot, — the mouth or buccal pouch, — while the rest of the chemical remains some distance away. It is obviously impossible for the animal to orient itself in accordance with the natural lines of direction of the diffusing ions. If the organism turns away from the side affected by the chemical, it will of course turn toward the aboral side, — that opposite the mouth, without regard to the original direction of the source of diffusion of the chemical, — and this is exactly what the animal does.

Parallel conditions exist in the other infusoria. In Paramecium, for example, a strong current, corresponding to that which reaches the buccal pouch in Stentor, passes along the oral groove to the mouth, the current over the rest of the body being slight in comparison. When a colored solution is used, and a nearly or quite quiet Paramecium is found, it may be observed that an advancing chemical behaves in much the same way as in Stentor. A cone of the solution is drawn out opposite the anterior end of the Paramecium, and passes down the oral groove to the mouth (Fig. 9). The Paramecium receives its stimulus from the chemical, therefore, on the oral side, —and responds, like Stentor, by turning toward the aboral side, — usually after swimming backward some distance.

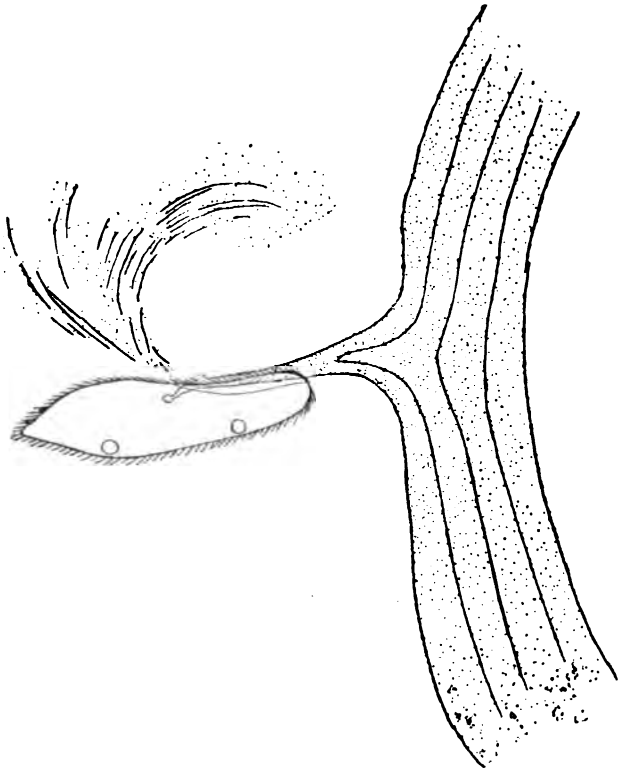

Figure 9. — Paramecium, showing how an advancing chemical is drawn out by the alimentary vortex, so as to reach the oral side without affecting the rest of the body.

These examples show that we are not justified in expecting the ciliate infusoria in which similar conditions occur to orient themselves to the lines of direction of diffusing ions, as presupposed by some current theories of the reactions of organisms to chemicals. The organisms are active, and determine for themselves where the stimulus shall first affect them. It is not at all surprising, therefore, that they have not been found thus to orient themselves.

In the Flagellata, owing to the minute size of the body, it is impracticable to determine by experiment whether the conditions for stimulation are or are not the same as those just described for ciliates. But in view of what is known of the movements of the flagella in these organisms, with resultant formation of a vortex having its apex at the mouth,[1] together with the known asymmetry of most flagellates, it can hardly be doubted that the conditions are practically identical with those found in the ciliates. In this group, then, as in the Ciliata, we should not expect to find the organisms orienting themselves to the lines of diffusing ions; they do not permit the ions to follow alone the laws of diffusion, but actively intervene to determine the distribution of the substance in solution. These facts certainly deserve consideration in all work on the reactions of Ciliata and Flagellata to chemicals.

Similar considerations apply to the reactions to other stimuli, in so far as the distribution of the agents concerned depends upon currents in the water. This would be the case, for example, with the reactions to heat and cold, in so far as the stimulation is due to differences in the temperature of the water in different regions. Figures 8 and 9 would serve equally well for the conditions when we have an advancing region of water which is warmer or colder than that about the infusorian. The warmer (or colder) water would be drawn out into a cone and then into a stream, which would affect only the oral side of the animal. It is therefore not surprising that we do not find a direct orientation produced by heat and cold in these animals, the so-called thermotaxis being brought about through the mediation of the "motor reaction" (backing and turning toward the aboral side; see Jennings, 1899*a*, page 334).

To radiant heat, to light, and to the electric current, these considerations, of course, do not apply, as the distribution of the stimulating agent in these cases is not affected by currents in the water.

The fact that Stentor and Paramecium (as well, of course, as many other infusoria) are first stimulated by a chemical on the oral side, and that they respond by turning toward the opposite (aboral) side, seems to indicate that the reaction of these organisms is, primitively at least, truly a localized one. The reason why in reacting they always turn toward the same side would be merely because they are always stimulated on the same side (the opposite one). If this is true, we should expect them, if the stimulus were in some way made to affect the other (aboral) side, to turn toward the oral side, contrary to their usual habit. This may have been the original condition of affairs, and possibly infusoria may exist in which it is realized even at the present time. But that it is not true for most of the infusoria is shown by the reactions to localized mechanical stimuli, as described in the fifth of those studies (Jennings, 1900). It there appears that when ciliates are stimulated on the (unaccustomed) aboral or right side, they respond by turning toward that side, — exactly as when they are stimulated on the opposite side. The unilateral method of reaction has become strongly stamped upon the organisms, being indicated in the unsymmetrial form.

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[1] See DELAGE et HEROUARD, 1896, pages 306–312, for a full account of the movements of the flagella and the formation of the alimentary vortex.

III. Osmotic stimuli. — As in the case of Paramecium, sugar seems not to affect Stentor through its chemical qualities, but only through its osmotic action, so that opportunity is given for determining the nature of the reaction to changes in the osmotic pressure of the surrounding medium. $\frac{m}{30}$ cane-sugar (about 1 per cent) caused no reaction whatever, though electrolytes of the same osmotic pressure caused a marked reaction, — showing the effect to be due to the chemical qualities, in the latter case. When Stentor was flooded with $\frac{m}{10}$ cane-sugar, there was no reaction for seven or eight minutes. By this time the plasmolyzing effect of the solution was very evident; the animals had shrunk considerably. Now there was a sudden strong contraction, the animal remaining contracted several minutes. It then let go its hold and abandoned its tube, forcing its way backward out of the latter.

Even with $\frac{m}{1}$ sugar (about 34 per cent) the response was not immediate. The animal conducted itself normally for about twenty seconds after it was flooded with the solution. By this time shrinkage due to plasmolysis is very evident to the eye; the animal contracts and finally leaves the tube.

B. OTHER FIXED INFUSORIA.

In giving an account of the behavior of some other fixed infusoria, I shall confine myself largely to a comparison with Stentor rœselii, bringing out the resemblances and differences, and entering into details only in case of important differences or additional features.

STENTOR CÆRULEUS EHR.

Stentor cærulus differs from S. rœselii in form and in its blue color, and it is usually larger, at least in this region. It does not inhabit a tube, and though frequently attached, it is much more inclined to a free life than is S. rœselii, so that it is often found swimming freely in large numbers.

In an attached Stentor cæruleus the ciliary currents are essentially like those of S. rœselii, and parallel statements may be made for both species as to the ingestion of food particles.

Stentor cæruleus is much more sensitive than S. rœselii; otherwise its reactions to mechanical and chemical stimuli are of the same general character, though with several important points of difference.

Stentor cæruleus does not usually bend over toward a solid object touching one side of the disc, as does S. rœselii. A large number of experiments on this point gave uniformly negative results.

When the particles of solid substance which are brought against the disc by the water currents are too large, too numerous, or mingled with some chemical, the animal responds, as does S. rœselii, by twisting somewhat on its long axis, then bending toward the right aboral side into a new position; by reversing the ciliary current for an instant, and repeating this; by contracting the body; and finally by breaking from the point of attachment and swimming away through the water. In these reactions there is little that is essentially different from the corresponding reactions of S. rœselii. A favorite method of changing the position when stimulated is shown in Fig. 10. The specimen backs strongly until the stalk is doubled near the foot. The animal then straightens the body into the position

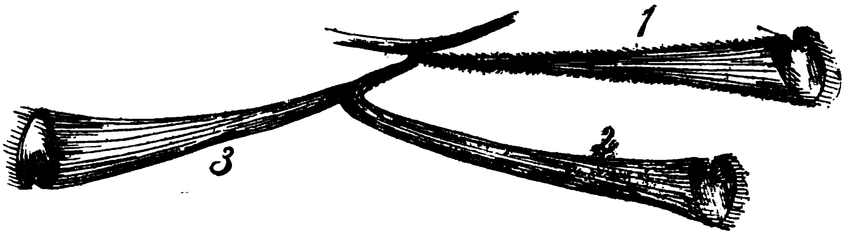

FIGURE 10. — Method by which Stentor cæruleus often changes position when stimulated. The animal occupies first the position 1, then pushes backward into the position 2, with stalk bent, then straightens into position 3.

indicated by the part next to the foot, — thus at an angle of 180° with its previous one.

Stentor cæruleus has recourse to the last step in the series of reactions, — the abandonment of its place of attachment, — much more readily than does S. rœselii. In the latter species I was unable to force the animal to leave its place by mechanical shocks alone· Specimens were stimulated by striking the disc with a glass rod, sometimes for an hour continuously, yet the animals did not leave their place. Stentor cæruleus, on the other hand, will sometimes break its attachment the first time it is struck with the rod. There is much variation among different specimens on this point; usually it requires many such strokes to produce the result, and all the other reactions in the series are tried first.

After leaving the place of attachment, Stentor cæruleus swims through the water in a rather wide spiral, revolving to the left. The body is usually somewhat curved toward the oral side, and apparently as a consequence of this, the animal swerves continually toward the oral side. The tendency to deviation thus caused is corrected by the revolution on the long axis. When the free-swimming Stentor receives a mechanical or chemical stimulus, it swims backward a little, then turns toward the right aboral side. The behavior of the free-swimming Stentor is thus essentially similar to that of Paramecium.

As the Stentor swims about well extended, frequently protoplasmic projections may be seen extending from the tip of the foot. These are viscid, so that bits of débris stick to them and are dragged about; sometimes other infusoria, such as Paramecium, coming in contact with the foot, are thus dragged along. I have seen two Stentors become attached to each other through the accidental coming together of the two posterior tips. If a small glass rod is placed against the tip of the foot, the Stentor may frequently be dragged backward by it, owing to adhesion.

Often Stentor drags its foot over the bottom or over pieces of plant material. Sometimes it stops in such a position, and in a few seconds the foot is securely fast and the animal is anchored anew.

Stentor cæruleus, unlike S. rœselii, reacts to light. The reactions of this and some other ciliates to light will be treated in a separate paper.

VORTICELLA.

Owing to its minute size, Vorticella is much less favorable for a study of behavior than is Stentor. I was especially desirous of investigating it however in connection with certain statements made in a very interesting paper by Hodge and Aikins (1895). These authors investigated chiefly the question of the rhythmical character of the activities of Vorticella. They kept a single Vorticella under observation without a moment's intermission for a period of twenty-one hours, besides intermittent study for a number of days. The observations showed "that a Vorticella works continuously and shows in its life no period of inactivity or rest, corresponding to periods of rest in higher animals. In other words, a Vorticella never sleeps." During five days the cilia were in continuous motion, and food was continuously taken.

Incidentally, Hodge and Aikins made a number of observations on other points. One of these was in regard to the modifiability of reactions in Vorticella. An attempt was made to feed the individual under observation upon yeast plants, by introducing some of a pure culture of these organisms into the preparation. "This attempt resulted in an interesting demonstration of the educability of Vorticellæ. At first they took this, to them, newly discovered food with great avidity, filling their bodies to distention with food vacuoles of the yeast. In a very few minutes, however, the entire meal was ejected with volcanic energy. Not a single torula was allowed to remain in the body, and for several hours at least — how long the memory lasted was not determined — the individual could not be induced to repeat the experiment."

It is much to be regretted that further details are not given in regard to this interesting experiment. We are not told whether the Vorticella continued its normal behavior and took in other food during the time in which it refused the yeast. It might be that the animal was merely injured by the food, and took nothing more into its body until it had recovered. We are not informed in what way Vorticella refused later to take the yeast, — whether by contracting, by reversing the ciliary current and turning the yeast out of the pouch, or in some other way. Yet upon these points depends largely the interpretation that shall be given to the observation.

I have endeavored, and as I judge with some success, to reproduce the essential features of this experiment. I have not succeeded with the yeast, for the Vorticellæ at once contracted, in my experiments, when the yeast culture was introduced. But a similar result may be obtained in a very simple way. In the case of the yeast culture we have a fluid containing various chemicals in solution, and holding in suspension many small bodies. These conditions may be imitated by grinding up ordinary carmine in water. A little of the carmine goes into solution, as may be shown by filtering the water, which will be found to have become red. This red solution was found to act as a slight chemical stimulus, on both Vorticella and Stentor.

When some of this carmine and water is added to the water about the Vorticella, the course of events is about as follows. For a short time — ten to fifteen seconds, or sometimes more — the current caused by the cilia is kept up in the usual manner, and many of the carmine grains are taken into the internal protoplasm, forming red food vacuoles. Then there is a sudden contraction of the stalk, the ciliary disc closing at the same time. This is repeated several times, the ciliary disc however remaining closed while the stalk partly extends and recontracts. Then usually the Vorticella extends in a new direction. If the carmine continues to be present, the contractions are repeated for ten or fifteen minutes or more. Then the stalk may remain extended, but the ciliary disc remains closed, so that no more carmine is ingested. This condition lasts as long as the carmine is present in large quantities.

Thus in this case, as in that described by Hodge and Aikins, Vorticella at first ingests a certain substance which it later refuses. This is also true of Stentor, as will be seen by consulting the account above given of the behavior of Stentor when much carmine is added to the water containing it. In no case was Vorticella observed to throw out the granules which it had already ingested, as described by Hodge and Aikins, but this is perhaps an unessential difference, as of course this has nothing to do with the "educability" of the animal.

In this experiment, I am convinced that the refusal of the Vorticella to continue to take the substance is due to a too great stimulation, either in the quantity of material, or, more probably, in the strength of the chemical action of the substance, rather than to any precise choice in the kind of substance ingested. There is evidence for this in the following fact. If the quantity of carmine in the water is greatly decreased, so that only scattered grains are left, the

Vorticella or Stentor no longer reacts to these, and they are ingested, gradually forming red vacuoles in the endosarc. Whether this would have been true in the experiment of Hodge and Aikins the data they have given do not enable us to judge.

In regard to a point closely connected with the above, the attitude of Hodge and Aikins must, I think, be considered uncritical. This relates to the sorting of food by the cilia. Among the "psycho-reflexes" of Vorticella, Hodge and Aikins include "3. Sorting of particles by the sensory cilia; the driving of food toward the mouth, and the driving away of waste particles." Further "When a particle is touched by the cilia an act of choice is apparent, and in accordance with this choice the particle is carried toward the mouth or whirled away." "Particles scarcely visible under the microscope are sorted with the greatest apparent precision." "A prime condition of the creature's life must be ability to distinguish food from that which is not food."

Beyond these general statements quoted no details are given. The authors report no critical observations or experiments as to what substances are ingested, what rejected in this sorting process. It is well known that investigators that have made such experiments have concluded that no such sorting takes place. Thus Verworn (1889, page 150) found that Vorticella ingests carmine grains, indigo, and chalk crystals, and I have myself observed the same facts. These are substances which cannot serve as food, so that Hodge and Aikins are certainly mistaken in their belief that "a prime condition of the creature's life must be the ability to distinguish food from that which is not food." Such organisms as Vorticella and Paramecium grow and multiply in situations such that the substance brought to the mouth by the currents consists largely of food, without any sorting; when this condition disappears, the organisms quickly die. The ingestion at the same time of some substances which do not serve as food is not particularly injurious to the organism, as these are simply passed out of the body with the waste matter at the time of defecation.

The impression that a sorting and selection takes place among the particles brought to the mouth probably arose from the following observations. Vorticella, as well as Stentor, brings to the buccal pouch in its alimentary vortex many more particles than are taken into the body. When the water contains many particles a continuous stream of these may be seen passing out of the buccal pouch. From this it is most natural to conclude that the material has been sorted, the valuable particles ingested, and the particles which are not nutritious turned away. But experiment does not support this conclusion. Thus, when all of the particles are of the same sort, either nutritious or not, part are taken into the interior, while a large portion are turned away. This is true on the one hand of grains of sepia, which are quite insoluble and non-nutritious; it is true, on the other hand, also, when many nutritious unicellular algæ are brought to the mouth. In the latter case, as in the former, many more of the particles are turned away than are taken in. The explanation of these facts is evident when one considers ,the mechanism of the alimentary vortex, as has already been pointed out by Verworn (1889). A large, strong current of water is carried toward the disc of the Vorticella. Inevitably, much of the water misses the disc completely, and the food particles which it contains never touch the animal. Another portion of the water strikes the disc, but not all of this can enter the relatively small buccal pouch, so that many of the food particles which strike the disc are whirled away again into the water. In the same way a rapid whirlpool is formed in the buccal pouch, but only a small part of the water in this can reach the relatively minute mouth, and it is only from this small part that the food can be taken. Thus there is a continual stream of particles passing out of the pouch, that have never come in contact with the mouth.

These mechanical considerations explain also the following noticeable fact. When the water contains but few particles, whether nutritious or not, only a few are ingested, while a large proportion of them are whirled out of the pouch and away. When the number of particles in the water is great a large number are ingested in a short time, without regard to whether they are or are not useful as food. The number of particles ingested depends primarily upon the number which reach the mouth opening, and this is only a small proportion of those involved in the general ciliary vortex.

This question of the sorting power of the cilia is, I take it, merely one of fact, and not one involving any important principle. What we know of choice in the Rhizopoda, and the parallel phenomena in inorganic fluids, to which reference has already been made, shows that there would be nothing new in principle if the cilia of Vorticella exercised choice in the same way. But the facts seem to indicate that they do not. Choice in these animals seems to be shown only in such phenomena as the reversal of the ciliary motion, bending over into a new position, and contraction, — these being, of course, different methods, somewhat crude perhaps, of rejecting certain things, and thus of exercising choice.

C. Becoming Accustomed to Stimuli.

Are the reactions of such organisms invariable, or does the reaction to a given stimulus depend on previous subjection to the same or different stimuli? The problem of the modifiability of the reactions of these lowest organisms is one of great interest, but one on which there exists but little precise experimental data. Scattered allusions to changeability in the reactions of the lower organisms are to be found in the literature, especially with relation to what might be called acclimatization to stimuli. Massart (1901, page 8) states that it is often to be observed that organisms which have reacted several times in succession, at short intervals, to a given stimulus, lose, little by little, the power of responding to this stimulus, but that this is doubtless to be attributed to fatigue. Loeb (1900, page 228) has discussed such a case in the reactions of worms to a shadow, as described by Nagel, and has attributed the lack of reaction when the stimulus is repeated to "a simple after effect of the stimulus, a case that we often meet with in the physiology of both animals and plants." Davenport (1897, page 108) gives an example drawn from the behavior of one of the organisms at present under consideration. "When an organism has been stimulated by contact for some time, it at last becomes changed, so that it no longer responds as it did at first. Thus, Dr. W. E. Castle informs me that he has seen a colony of Stentors, in an aquarium, being constantly struck by Tubifex waving back and forth, yet the Stentors did not contract as they usually do when struck."

Such contractions in the fixed infusoria furnish a most favorable opportunity for an investigation of this matter, and I therefore undertook to obtain some precise experimental data upon the subject. Experiments were made upon Stentor rœselii, S. cæruleus, Vorticella, Epistylis, and Carchesium.

First, the conditions described in the observation by Castle, above cited, were imitated, by striking the extended infusorian with a fine glass rod or hair, under the Braus-Drüner stereoscopic binocular. The chief difficulty in these experiments is to make the successive strokes approximately equal in force. This can be done but very imperfectly; nevertheless the results are clear.

The first stroke, whether light or heavy, given to an individual that has been undisturbed for an hour or more, almost invariably results in causing a quick contraction. This is true for all the organisms worked with. The animals remain contracted a minute or less, then slowly extend. At the instant when extension was complete, another stroke was given. This, and several successive strokes usually caused the same reaction as the first one. After ten or a dozen reactions, however, the organisms usually did not contract as soon as touched; the stroke had to be repeated one or more times before reaction was caused. A typical series for Stentor cæruleus is given in the following. The figures represent the number of strokes in each case before contraction took place, — a contraction occurring thus at each dash:

1—1—1—1—1—1—1—2—2—1—2—1—2—4—1—1—1—1—1—2—6—10—1—2—9—13—3—14—7—3—2—3—3—9—18— (at this point the Stentor pulled its foot loose and abandoned its place).

As is evident from the above, there is much irregularity in the number of strokes required to cause contraction. This is due, partly at least, to the practical impossibility of giving successive strokes of equal force. But the Stentor responded at first to the lightest possible touches, while later it required a considerable number of smart strokes to cause contraction.

Sometimes there is a ready response only to the first touch, as in the following series (Stentor cæruleus):

1—22—25— (breaks away).

1—1—40— (breaks away).

In these cases the organism does not remain entirely oblivious to the blows, but after it has ceased to react by contracting it continually changes its position, by twisting, then turning toward the aboral side, as if trying to escape from the blows. The final reaction, in Stentor cæruleus, is to break away from its attachment and swim away.

In Stentor rœselii, Vorticella, Epistylis, and Carchesium, similar results were obtained, save that these organisms never broke away from the attachment as a result of such mechanical stimuli. A typical series for an individual of Epistylis flavicans, var. procumbens, was as follows:

1—1—1—1—1—1—1—2—33—25—7—13—36—20—14—13—13—33—9—30—3—31—226.

In another series the results were as follows:

1—22—10—3—3—1—1—22—59—125— (continuous blows for 1 min.) — (¾ min.) — (1½ min.) — (4½ min.).

Some series show greater irregularity than the above. As in the case of Stentor, during the latter part of the experiment the Epistylis continually changed its position, as if trying to escape from the blows.

A typical series for Stentor rœselii, obtained in this case by jarring with the rod the leaf to which the Stentor was attached, is as follows:

1—1—1—1—1—1—1—1—1—1—1—1—3—1—5—1—1—3—1—3—3—48—40—2—250—36—36—154.

The results for Vorticella are similar. In a typical case the animal contracted after each of the first nine strokes. Then the contractions became less sudden; two or more strokes were required to produce them. After about twenty contractions the Vorticellæ could be tapped almost indefinitely without causing further contraction.

Carchesium polypinum is a tree-like colony composed of many Vorticella-like individuals, attached to the branches of a common stalk. The stalk muscles of the individuals are not continuous throughout the colony, so that it is possible, though not usual, for each individual to contract separately.

Carchesium shows very markedly the acclimatization to a stimulus. Observing first the reactions of a single individual that is repeatedly stimulated, it is found that its stalk contracts strongly at every stroke. But after about five minutes there is a marked change in the readiness to respond. Several strokes are required to cause contraction. Still later the stalk ceases to contract when the individual is struck, though for a time the peristome is folded inward and the ciliary motion ceases after every stroke, without contraction of the stalk. When thus continuously stimulated, usually the stalk contracts at intervals of two or three minutes, — though the strokes come as often as one per second.

The effect of the stimulation of a single individual on the colony as a whole is interesting If a single individual in an otherwise undisturbed colony is struck with the glass rod, usually the entire colony contracts at once, forming an almost solid ball. Apparently the sharp contraction of a single individual, by jarring the colony, acts as a stimulus to cause the contraction of all the other individuals. If the stimulus is repeated (on the same individual) as soon as the colony has become extended, usually only about half of the colony contracts. The third time only the large branch reacts to which the individual stimulated belongs. After this the number of neighboring individuals contracting when the single individual reacts is variable, ranging usually from half a dozen to thirty or forty. When the condition is reached where the individual, continually stimulated, reacts but once in two or three minutes, nearly the entire colony contracts with it.

What is the explanation of this failure to react to a stimulus to which the organism at first reacts readily? Three possibilities present themselves. (1) The lack of reaction might be due to fatigue of the contracting apparatus (corresponding to muscular fatigue in the higher animals). (2) It might be due to fatigue of the sensory function, so that the organism no longer perceives the stimulus (corresponding to fatigue of the sense organs in higher animals). (3) It is possible that the phenomenon cannot be explained as fatigue, so that all we can do is to formulate the facts, calling it an "after-effect," or other name which carries no implication as to its nature. We should perhaps have parallel phenomena for this also in the case of a higher organism, which reacts to a sudden, unexpected shock, but does not react a second time, though the stimulus is repeated, and is perceived by the organism.

Some farther data needed for forming an opinion as to which of these possibilities represents the truth may be obtained by varying the experiments. Striking the animal with the glass hair is a rather brutal method of experimentation; reactions may be produced with much slighter stimuli, and the results are much clearer.

For this purpose weak currents of water may be employed. This was done as follows: A tube 28 cm. long and of 5 mm. bore was drawn to a very fine capillary point and then filled with water. When the capillary end is below, there is of course a slight current of water from the tip, due to the pressure of the water in the tube above. Now the tip was brought close to an individual of Epistylis, so that the current flowed against the latter. At once the animal contracts. If the current is continued the Epistylis soon unfolds, and continues open and active in spite of the current. If now the tube is removed, so that the current no longer acts, then in a few seconds is restored, the animal does not react. Moving the tip of the tube over to a fresh specimen, this reacts at once. Moving it back to the first specimen, this does not contract. With a large colony of Epistylis, it was possible thus to test many specimens;

invariably the animal reacted to the stimulus of the current the first time, but later did not. In a very few cases a certain individual would react also to the second or third or even fourth stimulus, but soon ceased, and in a large majority of cases the animals reacted only the first time.

In Stentor rœselii the same results were obtained. The animals invariably reacted to the first stimulus of the current, but none of the numerous individuals studied reacted to a repetition. Stentor cæruleus behaves in a similar manner. In this species the individuals often respond only once by contraction, even to the stimulus of a stroke with the glass rod; after the first contraction they react only by bending over into a new position.

A large colony of Carchesium polypinum was situated just beneath the surface of the water. Touching the surface film with a needle, the colony at once contracted strongly. It was allowed to expand, and the surface film touched as before. There was no contraction. Repeated touching of the film caused no reaction, except the first time. Jarring the branch to which a colony was attached gave rise to a parallel series of phenomena.

From these results it is clear that the lack of reaction cannot be due to fatigue of the contractile elements. It is possible, as I have demonstrated by experiment, to keep Stentor continuously contracting for an hour at a time. Yet the animal responds only once to a weak stimulus; it cannot be supposed to have been so fatigued by this single contraction that it cannot contract farther.

It seems evident also that the failure to react after the first time cannot be due to fatigue of the sensory or perceptive power. It can hardly be supposed that a single stimulus would result in such fatigue that further stimuli are no longer perceived. Moreover this supposition is directly negatived by the fact that in many cases there is other proof that the organism does continue to perceive the stimulus. Thus, with Stentor cæruleus, as described above, at the first stimulus by tapping with the glass rod the animal contracts suddenly and strongly. After this it no longer contracts, but the fact that it perceives the stimulus is shown by its bending far over first in one direction, then in another, as the stimuli are continued, as if trying to avoid the blows. The impression made on the observer is very much as if the organism were at first trying to escape a danger, and later merely trying to avoid an annoyance. Similar phenomena may be observed with Epistylis and Vorticella.

Thus the third alternative seems the only conclusion to which we can reasonably come, in view of the facts. The organism becomes changed after stimulation, in such a way that it no longer reacts to a stimulus to which it at first reacted. There is a difference in the physiological condition of the organism before and after the stimulus. One can hardly avoid comparing these phenomena with the fact that in a higher organism a sudden unexpected touch or other stimulus will cause a reaction or "jump," when the same stimulus, not unexpected, causes no reaction whatever. It seems not improbable that the phenomena are similar in fundamental character in the two cases.

This resemblance is increased by certain further considerations. It is only when the stimuli are non-injurious that the unicellular organism ceases to respond upon repetition of the stimulus. If the stimulus is very powerful or injurious, the reaction is continued indefinitely. I attempted to accustom Stentor to the stimulus from a very minute quantity of $\frac{m}{150}$ NaCl, brought close to it with a minute capillary tube. Though the stimulus was repeated at very short intervals for an hour steadily, the Stentor reacted in every case; there was no indication of becoming accustomed to the stimulus.

The changes to be observed in the character of the reactions to a given stimulus when repeated show the same relation to the nature of the stimulus. As described in the first part of this paper, when the stimulus continues, and is powerful so that the reactions also continue, the reaction does not remain the same; but there is a series of different reactions. This series is a progression from less effective to more effective reactions, culminating in the animal's abandoning its place. On the other hand, as we have seen above, the reaction is sometimes changed also in the case of a weak stimulus, as when Stentor is tapped with the glass rod. But in this case the progression is in the opposite direction, — from a strong, effective reaction (contraction) to a weak one (bending over to one side.) The course of the reaction series, whether from less intense to more intense, or vice versa, depends upon the nature of the effect of the stimulus on the organism.

D. ANALYSIS OF THE OBSERVATIONS, WITH DISCUSSION OF THEIR BEARINGS ON CERTAIN GENERAL PROBLEMS.

The examination of the behavior of Stentor shows a striking contrast with the known behavior of Paramecium in the much greater complexity and adaptability of the former. In Paramecium the behavior seems made up of a few simple reflexes, with little variation or adaptability. In Stentor, on the contrary, this is far from being the case. This difference is due, I believe, to the different method of life. Paramecium is typically a free-swimming organism. As I have pointed out elsewhere,[1] in the open water there are few stimuli to guide an organism, the conditions being nearly uniform in all directions. Especially is this true in the case of an organism which, like Paramecium, is not sensitive to light. The result is the development of a few simple, almost machine-like devices for governing locomotion. Such a device is the spiral course, preventing the organism from aimless wandering in circles; such a device is the invariable turning toward a certain structually marked side when stimulated, which is so striking in Paramecium. On the other hand, an organism on the bottom is continually receiving stimuli of varied character, and it develops in consonance therewith a varied behavior. This difference between the behavior of free-swimming organisms and that of those which live on the bottom is very great, and its importance is not usually recognized. Even in the same individual the behavior becomes of a very different type on changing from one of these situations to the other. Stentor when free-swimming has the same simple behavior shown in Paramecium, while in Paramecium and other infusoria the behavior is greatly modified by contact with surfaces.[2]

Proceeding to an analysis of the behavior of Stentor, it is evident in the first place that the same external stimulus is not always answered by the same reaction, but that the reaction given depends largely on the history of the individual (and thus upon its present physiological condition). Thus we find the following to be true: —

1. After reacting to a given stimulus one or more times, if the stimulus is not a harmful one, the organism may cease to react, though the stimulus is repeated without change.

2. After reacting to a given stimulus the first time by a very pronounced reaction (contraction), the organism may later react, if the stimulus turns out to be a non-injurious one, by a very slight reaction, as by bending over to one side.

3. In the case of a stimulus which must in the long run be classed

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[1] See JENNINGS, 1901.

[2] On some of the modifications in the behavior of organisms when in contact with surfaces, see especially PÜTTER, 1900, and JENNINGS, 1897, pages 305–312. There is opportunity for further investigation in this matter.

as harmful, as when a dense cloud of carmine is added to the water, a series of reactions is to be observed, becoming of more and more pronounced character, until by one of them the organism rids itself of the stimulus. The course of events in such a case is usually as follows: —

a. No reaction at first; the organism continues its normal activities for a short time.

b. Then a slight reaction by turning into a new position, a seeming attempt to keep up the normal activities and yet get rid of the stimulus.

c. If this is unsuccessful, we have next a slight interruption of the normal activities, in a momentary reversal of the ciliary current, — tending to get rid of the stimulus.

d. If the stimulus still persists, the animal breaks off its normal activity completely, by contracting strongly, — devoting itself entirely, as it were, to getting rid of the stimulus, — though retaining the possibility of resuming its normal activity in the same place at any moment.

This reaction is repeated many times, the organism extending and immediately re-contracting as soon as the stimulus is perceived. In this case it is interesting to note that the organism now responds at once to a stimulus (by contracting) to which it at first did not respond, or to which it responded only by a reaction of different, less decided character. In paragraph 1 above we have the opposite case, where the organism ceases to respond to a stimulus to which it at first did respond.

e. Finally, if all these reactions remain ineffective in getting rid of the stimulus, the animal not only gives up completely its usual activities, but puts in operation another set, having a much more radical effect in separating the animal from the stimulating agent. It abandons its tube, swims away, and forms another one in a situation where the stimulus does not act upon it.

It is to be noted that this series of reactions is not of such a character that each step necessarily produces the next one; on the contrary, the bringing into operation of any step depends upon the ineffectiveness of the preceding ones in getting rid of the stimulus. The series may cease at any point, as soon as the stimulus disappears.

Further, the succeeding reactions are not mere accentuations of the preceding ones, but differ completely in character from them, being based upon different methods of getting rid of the stimulus.

Throughout the whole of the series of reactions the stimulating agent remains without change. The differences in reaction are due then to changes in the organism, — to such changes as in a higher organism might be called changes in the "state of mind." Here we may perhaps call them changes in the " state of protoplasm," though without implying that the two expressions are fundamentally different in signification.

It is clear that it is impossible to bring such behavior under the rubric "tropisms" or " taxis," or to present it as purely reflex in character; we must at the very least take into consideration physiological states of the protoplasm, as well as reflex factors. To gain a really satisfactory insight into the behavior, it is necessary to go farther than this, and tò take into consideration the ends to be attained by the different reactions and changes in reaction, — though whether this necessity has its foundation only in the human way of looking at things, or is really inherent in the behavior of Stentor, is a question on which there may be difference of opinion. In any case it will be well to analyze the behavior a little farther from this point of view. So far as outward appearances go, Stentor seems to react, like a higher organism, not merely to a stimulus now present, but to what is to come, — to the results of the action, as well as to the present conditions. The changes in the reactions, as the stimulus continues, seem to be directed toward the end of getting rid of the stimulus, — a different method being tried when one method fails. In the method of formation of a new tube, the same apparent reference to an end to be attained is forced upon the attention; there is no visible stimulus for the backward and forward movement of the Stentor, which results in the formation of a new tube; no reason that can be seen for this movement, except that it does form a tube.

We have thus in this unicellular organism the outward signs of action directed toward the accomplishment of certain ends, and thus, in so far, of intelligent action. There are, of course, a number of different ways of interpreting such phenomena. To say that the reaction is really directed toward the accomplishment of an end, is to say that the animal reacts, not merely to a present external stimulus, but also to a non-present result of its reaction. This is only possible if the organism has already, at some previous time, experienced this result, so that the latter has left a trace; has modified the organism, — changed its physiological condition. The organism when stimulated reacts in accordance with, or in consequence of, this modification, as well as in response to the external stimulus; the result is action directed toward an end.

Thus in action directed toward the accomplishment of an end there is an element in the organism, — a " trace " or " modification," corresponding to the result to be attained, and due to previous experience of this result. But a different view is often taken of action which appears outwardly to be directed toward the accomplishment of a certain result. In many such cases it is maintained that the organism really has no trace or modification corresponding to the result attained. In the case of Stentor, it would be held that the organism has become a sort of mechanism which gives a definite series of responses, when energy of such and such a character acts upon it under such and such conditions, for such and such a period of time. The result follows just as a precipitate is produced in a chemical reaction. The difficult problem according to this view is how reactions happen to be produced that are adapted to the accomplishment of certain ends. This is explained (usually) by natural selection.

In many of the instincts of higher organisms, such a view as that last set forth seems forced upon us by the fact that the organism has had no opportunity to get impressed upon it any trace or modification corresponding to the result to be produced. The animal responds before it has ever experienced the result. Cases of this sort will occur to every one.

In Stentor, however, this difficulty perhaps hardly exists, since it is not possible to separate sharply the given Stentor from its ancestors that may have experienced the results of any given reaction. Since each Stentor arises by simple division of a previous Stentor, there is here no special difficulty in the inheritance of acquired characters. If a given Stentor has become modified by certain experiences, there is no evident reason why the two Stentors derived from it by division should not retain this modification. Hence we have no absolute ground for maintaining on this basis that in Stentor the apparent reaction with reference to the result to be attained is not really a reaction with such reference. In other respects, we seem to have the same problem in attempting to explain the behavior of Stentor that we have in the instincts of higher animals.

It may not be out of place, finally, to indicate the bearing of the behavior of Stentor on the problem of consciousness in the lower organisms, a matter which has been much discussed of late. I do not see that there can be any objective criterion of consciousness, hence this question in strictness does not fall within the field of an investigation directed to the end of determining what observation and experiment can tell us of the behavior of an organism. But it may be of interest to point out the relation of the phenomena described to certain questions that have been raised. In former papers (Jennings, 1899*a*, page 339; 1899*b*, page 13), I expressed the opinion that in Paramecium the behavior was comparable to that of an isolated muscle, and that " we are not compelled to assume consciousness or intelligence in any form to explain its activities." This statement is, of course, well within the facts, as far as objective investigation can give them to us, yet it is perhaps of little significance, since it could probably be made for any organism, outside of the self. The behavior of Paramecium is of a character to emphasize strongly the possible machine-like character of the activities of the lower organisms. In Stentor we have a very different case, showing that the behavior of Paramecium cannot be considered a type for that of all infusoria. Paramecium has become adapted in its behavior to a very simple set of conditions, and its behavior is of corresponding simplicity. In the behavior of Stentor, we find all the outward indications of action directed toward the accomplishment of certain ends. We have then the same ground for attributing consciousness to Stentor as to higher animals which show behavior of a similar character, — no more, no less.

In a recent paper Minot (1902) has expressed the opinion that " the function of consciousness is to dislocate in time the reactions from the sensations," — to inhibit the direct reactions at certain times; to cause reactions at certain times to stimuli that have occurred previously. " This disarrangement . . . seems to me the most fundamental and essential characteristic of consciousness that we know, — " " — and so far as we know, it belongs exclusively to consciousness."

Judged by this criterion (substituting " stimulus " for " sensation " as used by Minot) we should clearly have to attribute consciousness to Stentor. As shown above, this organism at certain times inhibits the reactions to stimuli, to which at other times it reacts strongly. Moreover, the nature of its reactions to a given stimulus depends upon stimuli previously received, and this I think is all we can mean when we say that an animal reacts at a certain time to a stimulus previously received. I confess that Dr. Minot's criterion seems to me by no means an absolute one; and that unconscious mechanisms could be constructed, and indeed do exist, in which there is a dislocation in time between the action of an outer agent upon the machine and the reaction of the machine, similar to that which we find in organisms. It is nevertheless interesting to find the behavior of a unicellular organism falling within the category that would be considered conscious by Minot.

If we consider now the criterion held by Loeb and Bethe, — that consciousness depends upon associative memory, upon the power of learning, — it is perhaps not so easy to decide where Stentor stands. The changes in reaction when the stimulus is long continued; first no reaction, then bending into new position, then reversal of the ciliary motion, then contraction many times repeated, and final leaving of the tube, could perhaps be considered cumulative effects of the stimulus, and hence as not giving evidence of associative memory. But this of course leaves quite out of consideration the fact these different reactions are all adapted, by different methods, to getting rid of the stimulus, and it is exactly this adaptation to an end that furnishes the real problem. How does the organism happen to give these particular reactions, thus adapted to the accomplishment of an end? If it gives these particular reactions as a result of experience, it has learned, hence, on the hypothesis we are considering, it has consciousness. If it has not learned to give these purposive reactions, the only alternative hypothesis as to how this has come about is, so far as I am aware, through the action of natural selection upon chance movements.

All together, it must be clearly recognized, I think, that objective study can give us nothing final on the problem of whether consciousness does or does not exist in the lower organisms. We can have indeed no absolute proof of the existence of consciousness outside of ourselves. Whether one holds that Stentor and Paramecium have or have not consciousness will depend chiefly upon his general system of philosophy, which is of course not mainly determined by observation and experiment.

E. SUMMARY.

The foregoing paper comprises a study of the behavior of Stentor rœselii, Stentor cæruleus, and Vorticella.

Taking Stentor rœselii as the type, the following are the most important points brought out:

I. In the unstimulated Stentor the ciliary motion causes a vortex whirling to the left and descending to a point on the left oral side. Only a small part of the water or suspended material in the vortex reaches the mouth. The unstimulated Stentor does not contract.

II. Under the influence of slight mechanical stimuli, as when carmine grains or other small objects are carried to the disc by the vortex, the behavior is as follows: —

A. For a time the normal behavior may be continued, some of the particles being ingested.

B. In some cases the Stentor may bend toward an object touching one side of the disc (" positive thigmotaxis ").

C. With repeated weak stimuli, the Stentor may react the first time by contraction; then cease to react farther, though the stimulus is continued. Or the animal may react the first time by contraction; later by merely turning to one side, as described in paragraph 1, below.

D. When the objects striking the Stentor are very numerous or large or are combined with a chemical stimulus, or are otherwise unfavorable, there is a series of reactions, as follows: —

1. The Stentor first bends into a new position, by twisting on the long axis, then bending toward its aboral side. It may thus rid itself of the stimulus; if not, this reaction is usually repeated a number of times.

2. If the reaction described above does not rid the animal of the stimulus, it next reverses the ciliary current for an instant. This reaction may be repeated a number of times.

3. If the stimulus still continues, the animal next contracts into its tube. This reaction is repeated many times, if the stimulus continues, and usually the period during which the animal remains contracted becomes longer.

4. Finally, if the stimulus continues, the animal lets go its hold and abandons its tube. It swims away through the water, its behavior while free being similar to that of Paramecium. After a time it forms a new tube, by a peculiar process, in another place, where it is not affected by the stimulus, and remains there.

III. Under chemical stimuli (1) the reactions are essentially the same as above described for mechanical stimuli, the series 1–4 described above taking place in a similar manner. (2) The distribution of a diffusing chemical approaching the Stentor is determined by the ciliary vortex of the Stentor. The result is that the chemical arrives at the mouth and oral surface of the Stentor before it touches any other part of the body; the latter may remain for a long time quite unaffected. Hence the conditions necessary for the orientation of the body in lines of diffusing ions are not present, and such orientation cannot occur. This is true also for other ciliate infusoria; probably also for flagellates.

Similar considerations apply also to the reactions to temperature variations in the water.

IV. To osmôtic stimuli Stentor responds only after plasmolysis is far advanced.

V. In Stentor cæruleus the behavior is essentially similar to that of S. rœselii, — the same series of reactions being given to continued stimuli. The following differences are to be noted: —

1. Stentor cæruleus does not bend toward a weak mechanical stimulus at one side of the disc, as does S. rœselii.

2. Stentor cæruleus has no tube and abandons its place of attachment much more readily than does S. rœselii.

VI. In general the reactions of Vorticella are similar to those of Stentor, though it was not observed to abandon its place as a response to stimuli.

VII. Stentor, Vorticella, Epistylis, and Carchesium were found to become accustomed to repeated mechanical stimuli, so that they cease to respond by contracting when the stimulus is repeated. This is not due to fatigue, since they frequently respond only to the first stimulus. It is likewise not due to lack of perception of the stimulus, since after ceasing to contract they often give other evidence that the stimulus is perceived.

VIII. On the whole the behavior of Stentor is complicated, as compared with that of Paramecium, and shows considerable power of adaptation. Whether the animal reacts to a given stimulus or not, and how it reacts, depends upon previous subjection to this stimulus, and upon the previous method of reacting to it. If a stimulus continues, the animal gives a series of reactions which are not invariable in order or length of continuance; each reaction of this series is adapted, by a different method from the others, to getting rid of the stimulus. These reactions, together with the method of forming a new tube, have the appearance of being directed toward the accomplishment of definite ends.

LITERATURE CITED.

DAVENPORT, C. B.
1897. Experimental morphology, i.

DELAGE et HEROUARD.
1896. Traité de zoologie concrète, i.

HODGE, C. F., and AIKINS, H. A.
1895. American journal of psychology, vi, pp. 524–533.

JENNINGS, H. S.
1897. Journal of physiology, xxi, pp. 258–322.
1899*a*. This journal, ii, pp. 311–341.
1899*b*. American naturalist, xxxiii, pp. 373–389.
1900. This journal, iii, pp. 229–260.
1901. American naturalist, xxxv, pp. 369–378.
1902. Journal of applied microscopy, Jan., pp. 1597–1602.

JOHNSON, H. P.
1893. Journal of morphology, viii, pp. 467–556.

LOEB, J.
1900. Comparative physiology of the brain and comparative psychology.

MASSART, J.
1901. Annales de l'Institut Pasteur, Aug. 25.

MINOT, CHARLES S.
1902. Science, xvi, no. 392, pp. 1–12.

PÜTTER, AUG.
1900. Archiv für Physiologie, supplement-Band, pp. 243–302.

RHUMBLER, L.
1898. Archiv für Entwickelungsmechanik, vii, pp. 103–350.

VERWORN, M.
1889. Psycho-physiologische Protistenstudien, 218 pp.

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+ Jamieson, C.
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X. The movements and reactions
of pieces of ciliate infusoria.

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STUDIES ON REACTIONS TO STIMULI IN UNICELLULAR ORGANISMS. X. THE MOVEMENTS AND REACTIONS OF PIECES OF CILIATE INFUSORIA.[1]

H. S. JENNINGS AND CLARA JAMIESON.

The present paper records an attempt to investigate by operative procedure the division of labor among the organs of locomotion of the ciliate body ; to determine the part played by cilia of different position or structure in the usual movements and reactions. In many of the Ciliata the body is much differentiated, often bearing a number of different sorts of simple or compound cilia, variously distinguished as membranellæ, cirri, setæ, cilia proper, and the like. In such organisms as *Stylonychia* (Fig. 1) and *Stentor* these different structures have a characteristic distribution, and apparently differ markedly in function. Even in less differentiated infusoria a distinction between oral or peristomal cilia and body cilia can usually be made.

Observation of the behavior of these animals indicates that there is a division of labor among these cilia in the production of the usual movements. The organisms as they swim through the water follow a spiral course, usually swerving continually toward one side, but compensating the deviation thus caused by rotating on the long axis. The swerving is as a rule (though not invariably) toward the side away from the peristome or the oral cilia. The stroke of the oral cilia is of such a character as to tend to turn the body toward the side opposite to that on which they are situated, so that the swerving appears to be due to these cilia,—aided, as a rule, by the form of the body.

In reacting to most stimuli, these organisms turn toward a structurally defined side (see Jennings, 1900), and, as a rule, this is again the aboral side, or that opposite the oral or peristomal cilia. Observation indicates that this is due to the stroke of the oral cilia, or in some cases to a still smaller and more localized group of cilia. This has been set forth by a number of authors,—

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[1] Contributions from the Zoölogical Laboratory of the University of Michigan, No. 56.

notably by Pearl (1900), in the case of *Colpidium*, and by Pütter (1900, p. 273), in *Stylonychia*. In *Colpidium*, according to the observations of Pearl, it is a group of cilia at the anterior end of the body which, invariably striking toward the oral side when the animal is stimulated by the electric current, cause the body to turn toward the aboral side. In *Stylonychia* it is the entire group of peristomal cilia that, striking toward the oral side, cause the body to turn toward the aboral side.[1] We can testify from our own observations that these relations seem clear. One naturally concludes, therefore, that the sidewise motion is determined by these peristomal or anterior cilia, and that if they were removed the motion would no longer occur. Massart (1901, p. 27), on the basis of Pearl's work, accepts the idea that the sidewise movement is caused by different cilia from the backward or forward motion,—aiding thus to distinguish between what Massart calls *phobism* (the backward movement in reaction) and *clinism* (the lateral movement).

The work here set forth was undertaken for the purpose of getting further light on this and related matters. If the anterior or oral cilia are removed by operation, will the organism no longer turn toward the aboral side when stimulated? And is the character of the spiral swimming modified by removal of such cilia? In general, does the removal of specific groups of cilia (with the accompanying alteration of the body form) have specific effects on the movements of the animal?

The behavior of pieces of infusoria has been studied by Verworn (1889) and Balbiani (1888).[2] The results of these investigators are of the greatest interest, but do not touch specifically the questions raised above. They found that pieces of ciliates move in general in the same manner as do the entire organisms. The reaction to stimuli by turning toward a certain structurally defined side, and the swerving toward a structurally defined side in the spiral course, in the uninjured animal, had not at that time been observed, so that these matters were, of course, not observed for the pieces. The questions proposed in this paper were therefore not touched by the experiments of Verworn and Balbiani.

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[1] These are aided in the turning, according to Pütter, by the "running cilia."

[2] Balbiani's paper we have been unable to consult, so that we must depend upon the account of it given by Verworn.

In giving an account of the results of our experimentation, matters which were described by Verworn (1889) will not be dwelt upon, but the account will be confined to the points bearing on the questions raised above.

The operation of cutting the animals was done with a small knife, under the Braus-Drüner stereoscopic binocular, which has great advantages for such work. It was found best to cut the animals when resting against a clean glass surface. Most of the operations can be performed by the aid of some patience without great difficulty. In all cases the results set forth are drawn from experimentation and observation on a considerable number of specimens.

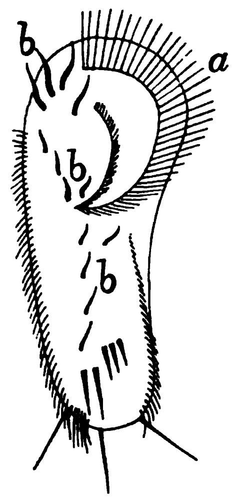

FIG. 1. *Stylonychia*, ventral surface, showing the distribution of locomotor organs (after Pütter, 1900).

1. *Stylonychia.*—Pütter (1900) gives a full account of the normal movements of this animal when uninjured, with a careful analysis of the action of the different cilia in producing the movements. For our purposes it is important to note the following different movements. (1) The animal runs forward on surfaces, chiefly or entirely by the aid of the "running cilia" *b*, Fig. 1. (2) When stimulated it jerks back a little, then turns to the right or aboral side. This turning appears to be due to the lateral stroke of the large peristomal cilia *a*. (3) The animal may also swim freely through the water; in doing so it swerves continually towards its right side, and revolves on its long axis from right over to left—the resulting path being a spiral. The swerving toward the right appears to be due to the lateral stroke of the peristomal cilia, and the revolution on the long axis might well be due to the same factor.

When *Stylonychia* was cut in two transversely just behind the mouth, so that the entire peristome remains with the anterior half, the pieces move as follows:

1. Anterior half, possessing peristome (Fig. 2, *a*).—After the first shock effects, the movement throughout is nearly like that

of the uninjured animal, but somewhat more rapid than usual. The piece runs forward on the bottom, turning to the right when stimulated. It differs from the entire animal in that it much more rarely jerks backward when stimulated, though it does this when the stimulus is powerful. When swimming through the water, it revolves to the right and swerves to the left, as in the entire animal. Since the anterior half possesses the peristome, the similarity of its movements to those of the uninjured animal was to be anticipated.

2. Posterior half, without peristome (Fig. 2, *b*).—Immediately after the operation the piece jerks rapidly backward, turning at the same time toward the right side. After a time it becomes quieter, and now it reacts essentially like the entire specimen, though it is somewhat less active. If stimulated with the glass hair at the posterior end its runs forward; stimulated at the anterior (cut) surface, it jerks backward and turns to the right. Chemical stimuli cause the same reactions. A little m/20 NaCl, or a weak solution of methylene blue was introduced with a capillary tube close to the piece; on coming in contact with the chemical the piece backs and turns to the right, just as is done by the entire animal.

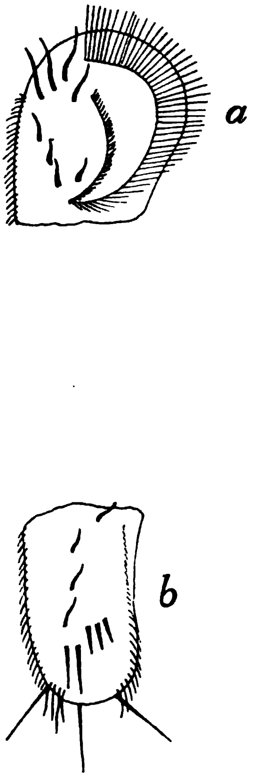

FIG. 2. Anterior (*a*) and posterior (*b*) halves of *Stylonychia*.

Thus the presence of the peristomal cilia is not the determining factor for the invariable turning to the right as a response to stimuli.

3. Posterior one fifth. — The movements are somewhat less well coördinated than in the larger pieces. But after the shock effects have ceased, the piece creeps forward, *turning to the right*. It swims slowly through the water, swerving to the right and revolving to the left. as in the uninjured specimen.

4. Anterior one third. — This is very rapid in its movements, and circles almost continuously to the right. In the free water it revolves in the usual way. When stimulated, it turns to the right, but *does not jerk back.*

5. Middle one half. — This was obtained by cutting off the anterior and posterior fourths of the body. Its movements and reactions are essentially similar to those of the uninjured specimen.

6. Right and left halves. — These were obtained by splitting *Stylonychia* lengthwise. They move and react (after the shock effects have ceased) in nearly the normal manner. Thus, when stimulated mechanically or chemically, they jerk backward, and the right half turns toward its right (uninjured) side, while the left half likewise turns toward its right (injured) side.

A specimen was cut lengthwise in such a way that the left piece was a strip comprising about one third the body. This moved somewhat irregularly, but reacted in the same manner as the left one half just described — though it evidently bore no part of the peristome.

7. A small, left posterior corner, comprising less than one fourth the animal, reacted, after the effects of the shock had ceased, in the usual way — by backing and turning toward the right.

Thus, on the whole, pieces of *Stylonychia* from any part of the body, if amounting in size to as much as one fourth to one half of the animal, move and react essentially like the entire specimen. In very small or very irregular pieces the movements become irregular, as might be expected.

Similar results were obtained with *Oxytricha fallax*, and with a number of unidentified *Hypotricha*, though the smaller size makes most other species less favorable for such experimentation than is *Stylonychia*.

II. *Stentor cæruleus.* — In *Stentor* the oral (or adoral) cilia or membranellæ form nearly a circle at the broad anterior end of the body. *Stentor*, when free swimming, follows a spiral path, revolving from right over to left. It reacts to chemical and mechanical stimuli by backing a little, then turning toward the right aboral side. It is evident to observation that the adoral cilia play a large part in the turning; from their large size and their position at one end of the body it would be natural to conclude that the turning is due to them alone.

1. The entire anterior disk, with the adoral cilia and the mouth, was removed by a transverse cut near the anterior end (Fig. 3). The posterior portion (*b*, Fig. 3), having only the body cilia, swam through the water as usual, in a spiral, revolving from right over to left. The aboral side in *Stentor cœruleus* is usually curved in a very different manner from the oral side, so that it is possible to distinguish the two sides even when the disk is removed. The specimen deprived of the disk and anterior cilia reacted to mechanical and chemical stimuli by backing and turning toward the aboral side, just as does the uninjured specimen.

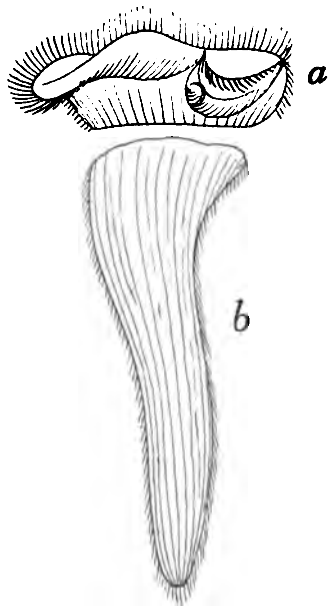

FIG. 3. *Stentor*, cut transversely just behind the disk.

After a time the specimen attached itself by the foot, and extended in the usual manner. Currents, due to the ciliary action, then passed backward toward the foot, or at times they were reversed, passing in the opposite direction. There was, however, no evident whirlpool formed, with a definite center, as in the uninjured specimen. The animal from which the disk had been removed, after becoming attached, reacted to mechanical stimuli by turning into a new position, and later by contracting, just as happens in the uninjured specimen. (On the behavior of *Stentor*, see Jennings, 1902.)

2. The disk portion (Fig. 3, *a*), bearing the adoral cilia and the mouth, also moved and reacted in the usual manner—revolving to the left as it swam, and responding to stimuli by backing and turning toward the aboral side.

3. Posterior one fourth—comprising about one half the slender "stalk" of *Stentor*.—This swims about, revolving to the

left. Whether on stimulation it turns toward a definite side could not be determined. After a time the small piece fixed itself by the foot and extended. The currents caused by the cilia were most of the time directed away from the attached end, being thus the reverse of the normal currents. At times, however, the current passed toward the foot.

When stimulated mechanically, the piece responded, like the entire *Stentor*, by bending into a new position, and later by contracting.

III. *Spirostomum ambiguum.*—Essentially the same results were obtained with this large infusorian as with *Stylonychia* and *Stentor*. Both the anterior half, bearing the adoral cilia, and the posterior half, without adoral cilia, move and react in much the same way as does the uninjured individual. They revolve to the left when swimming freely through the water, and turn toward the aboral side when stimulated. The anterior half moves more rapidly than does the posterior half, and when stimulated turns through a much greater angle than does the latter. The posterior half, if not strongly stimulated, frequently remains nearly quiet, or oscillates back and forth, as described in the third of these studies (Jennings, 1899). If stimulated mechanically or chemically, however, it backs and turns toward the aboral side.

A posterior one third of the body reacts in the same manner as the posterior one half.

A minute piece from the posterior end, less than half the length of the contractile vacuole, moved in the normal fashion, revolving to the left, and moving with the anterior (cut) end in front. The reactions to stimuli in such a small piece are very difficult to determine.

IV. *Paramecium caudatum.*—*Paramecium* is, of course, much less favorable for work of this character than are the infusoria of which an account is given above. This is due in part to the minute size and rapid movements of *Paramecium*; in part to the strong tendency for the cut pieces to disintegrate at once. But, with much patience, satisfactory anterior and posterior halves can be obtained.

1. Anterior half, bearing the oral groove and mouth (Fig. 4, *a*). This moves and reacts almost exactly as does the entire animal,—

revolving to the left, and turning toward the aboral side when stimulated mechanically or chemically.

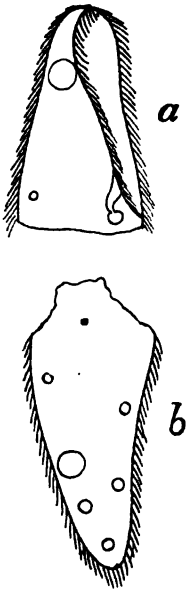

FIG. 4. Paramecium cut transversely. *a*, anterior half, with mouth and oral cilia. *b*, posterior half, without mouth or oral cilia.

2. Posterior half, without oral groove or mouth (Fig. 4, *b*). Much pains was taken to remove the entire oral groove and mouth, so that the posterior piece was usually a little less than one half the body. It was possible to distinguish the aboral side by the presence there of the contractile vacuole. The posterior half swims in a spiral, revolving, like the uninjured animal, to the left, and keeping the aboral side to the outside of the spiral. Its movements are slower than those of the anterior half, and it is much less sensitive, not reacting so readily to stimuli. The reaction to mechanical and chemical stimuli is by jerking backward and turning toward the aboral side, as in the uninjured individual. Thus the presence of the oral groove and oral cilia is not necessary in order that the usual movements and reactions may occur.

3. Middle third of *Paramecium* (anterior and posterior ends removed).—This behaves essentially like the anterior and posterior halves, or the entire animal.

Summary and Conclusions.

In all the Ciliata studied, including several of the Hypotricha (*Stylonychia*, *Oxytricha*, etc.), two of the Heterotricha (*Stentor* and *Spirostomum*), and one of the Holotricha (*Paramecium*), the movements and reactions of pieces of the organisms, if these are not too minute or too irregular in form, are essentially similar to those of the entire animal. The pieces swim in a spiral, swerving continually toward a certain side, just as do the entire organisms. They react to chemical and mechanical stimuli by backing and turning toward the same structurally defined side, as do the entire animals. This is true of pieces forming one fourth to one half the entire animal, whether taken from the anterior end, the posterior end, the right or left side, or the middle of the body. It is true, whether the piece does or does not bear any of the oral or other differentiated cilia.

These results establish the following facts:

1. The turning toward a structurally defined side after stimulation is not produced alone by the oral or peristomal or anterior cilia, or by any particular set of cilia, since it takes place in pieces of the body containing no specially differentiated cilia.

2. The swerving toward a certain side in the spiral swimming is not due alone to any special set of cilia, since it occurs in pieces from any part of the body.

3. The swerving toward a certain side is likewise not due to the geometrical form of the body (as to a groove on one side, such as we find in *Paramecium*), for it continues when the form of the piece is essentially altered (as by the removal of the oral groove in *Paramecium*).

4. The revolution on the long axis in a certain direction, while swimming, is not due to any special set of cilia, nor to any peculiarities of form of the body, since it occurs in pieces from all parts of the body.

5. From the foregoing paragraphs, 1–4, it must be concluded that the revolution on the long axis, the swerving toward a defined side in the spiral course, and the turning toward a structurally defined side when stimulated, are due to the method in which the cilia strike. This peculiar (one-sided) stroke is not limited to any particular set of cilia, but is shared by all the body cilia.

Division of labor is thus not marked, so far as these points are concerned, among the locomotor organs of these animals. As any portion of a crystal is organized like the entire crystal, so in the Ciliata any piece of the body, apparently, is organized so as to move and to react to stimuli in the same manner as does the entire animal.

LITERATURE CITED.

Balbiani, E. G.

'89 Recherches expérimentales sur la mérotomie des infusoires ciliés. Contribution a l'étude du role physiologique du noyau cellulaire. Recueil Zool. Suisse, T. 5.

Jennings, H. S.

'99 Studies on Reactions to Stimuli in Unicellular Organisms. III. Reactions to Localized Stimuli in Spirostomum and Stentor. Amer. Naturalist, vol. 33, pp. 373–389.

Jennings, H. S.

:00 Studies, etc. V. On the Movements and Motor Reflexes of the Flagellata and Ciliata. Amer. Journ. Physiol., vol. 3, pp. 229–260.

Jennings, H. S.

:02 Studies, etc. IX. On the Behavior of Fixed Infusoria (Stentor and Vorticella), with Special Reference to the Modifiability of Protozoan Reactions. To appear in the Amer. Journ. of Physiology, 1902.

Massart, J.

:01 Essai de classification des réflexes non nerveux. Extrait des Annales de l'Institut Pasteur. Aug. 25, 1901, 39 pp.

Pearl, R.

:00 Studies on Electrotaxis. I. On the Reactions of Certain Infusoria to the Electric Current. Amer. Jour. Physiol., vol. 4, pp. 96–123.

Pütter, Aug.

:00 Studien über Thigmotaxis bei Protisten. Arch. f. Anat. u. Physiol., Physiol. Abth , Supplementband, pp. 243–302.

Verworn, M.

'89 Psycho-physiologische Protisten-studien. Jena, 218 pp.

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TROPISMS

(1½)

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RAPPORT

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TROPISMS

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The theories grouped about the tropisms have long been the center of discussion; of criticism and defense. What are the tropisms? What part do they play in the behavior of lower animals? What have been the theories connected with them, what the grounds of criticisms, and how far are these justified? Is it possible at the present time to reach clearness, and perhaps agreement, on these questions?

What is the Tropism?

Tropism is an appropriate descriptive and collective term for classifying certain objective phenomena observed in the movements of organisms. *The tropism includes those reactions in which the organism takes and maintains a definite orientation — places the axis of its body in a definite position — with relation to some external source of stimulation.*

The tropism thus names an observed phenomenon, and certainly at first without any theoretical implications as to how it is brought about. The ground for separating off these *orientations* from the great mass of reactions, and giving them a separate name as *tropism* or *taxis,* is much clearer in a study of the actual phenomena than appears when one attempts to describe them in words. It is most striking to observe that under certain stimuli the organism takes up and retains a definite position, with its body in line with the direction from which the stimulating action comes, while under other stimuli this does not occur. The typical case is the position taken by many plants and animals when light shines upon them from a certain direction. The behavior then is so different from that shown by the same organism when a chemical or mechanical or temperature stimulus comes from one side that the need for a separate descriptive name is evident.

In accepting *orientation* as a chief criterion of the tropism, I am in agreement I believe with the other investigators who are to report on this matter; certainly with Loeb and Bohn. In a dissertation prepared under the direction of Professor Loeb we read « Loeb has pointed out that *every true tropism is due to an orientation of the organism* », and again « *Orientation* and *tropism are synonymous* expressions »[1]. Bohn explicitly accepts the tropism as defined by Loeb[2].

But it is of the utmost importance to recall the fact that an entirely different usage of the word is common. Many authors use the word tropism (or its equivalent, *taxis*) for all movements of advance and retreat, without any reference whatever to the existence of orientation. This is the usage of Pfeffer, one of the greatest of general physiologists; and in this usage Pfeffer is followed by a great body, both of botanists and zoologists[3]. This usage is perfectly justifiable if agreed upon: certainly no better authority on such matters can be found than Pfeffer. Pfeffer and his followers recognize that in the tropisms we have a most heterogeneous mass of phenomena, and it is their endeavor, by investigation, to break this mass into subordinate classes. The tropism is not defined *a priori;* how it is brought about is a question to be determined by observation, in each case. Thus we find various sorts of tropisms (or *taxis*) distinguished: phobotropism, topotropism, etc. etc.[4] Some of our best authors follow this usage in the study of the behavior of animals. For example, Washburn, in her recent admirable book « The Animal Mind » (1908) defines the tropism as follows: « The direct motor response of an animal to an external stimulus is known as a tropism » (p. 57).

Although I hold this very general usage to be entirely defensible, I shall not employ it farther here, save to point out how far authors may be at cross-purposes in using the word tropism in different senses. Discussion or criticism of theories as to the part played by tropisms in behavior, when the tropism is limited to orientations, or is otherwise rigidly defined, of course loses all point if one means by the tropism merely any « direct motor response of an animal to an external stimulus ». Misunderstandings based on this difference of usage have been common. Throughout the present paper I shall use tropism as signifying an orientation, as employed by Loeb, Bohn and others.

Employed thus as a collective name for all reactions in which a definite and continued axial orientation is a prominent feature. the term tropism is useful, and usually clear. It involves no theory, and there is no ground for criticizing its employment, when used in conformity with the facts; so far as I am aware, there has been no criticism of such employment.

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[1] Garrey, W. E. *The effect of ions on the aggregation of flagellated infusoria.* Amer. Journ. of Physiol., vol. 3, 1900, pp. 292 and 313.

[2] See Bohn. *La Naissance de l'Intelligence,* 1909.

[3] The present author formerly followed this usage.

[4] See Pfeffer, *Pflanzenphysiologie,* Bd. 2, p. 755.

Criticisms of Theories of Tropisms.

To understand the criticisms and discussions that have occurred, it is necessary to distinguish the *theories* of tropisms from the tropisms themselves. Tropism is merely the name for certain observed phenomena of behavior. The *theories* of tropism have dealt with the essential nature of these phenomena; with supposed uniform ways in which these observed phenomena are brought about; and with generalizations as to the part played in the behavior of lower organisms by the tropisms; they have necessarily, like all theories, outrun the facts. The criticisms have borne upon these theories, not upon the use of the word tropism for classifying certain facts.

Criticism of « the tropism theory », so far as the present writer is concerned, has been directed against the following: (1) Against the applicability of the tropism concept to certain concrete fields of behavior to which it had been (incorrectly) applied; (2) Against the uniform explanation of these reactions by certain simple processes; (3) Against judgments of the nature of reactions in lower organisms based too exclusively on the tropisms and their supposed uniform explanations; (4) Against overestimation of the part tropisms play in the behavior of lower organisms. Its main purpose was to bring out the complexity and regulatory character of behavior in lower organisms. and the great number of factors on which it depends, as against a theory of uniformity and simplicity.

Application of the Tropism Concept to Reactions that are not Tropisms.

It was natural, in the early stages of investigation, to generalize the methods of action seen in the tropisms; to hold that the reactions to other stimuli, not yet studied, were of the same sort. After discovering that in the reactions to light, to gravity, and to the electric current, orientation was a prominent feature, it was concluded that reactions to chemicals, to osmotic pressure, to mechanical stimuli, to heat and cold.

and the like, were of the same sort. Chemotropism, tonotropism, stereotropism, thigmotropism, thermotropism, and many other terms of the same sort were words commonly applied to these reactions; all were given a place in a general theory of tropisms, which was supposed to unify the reactions of lower organisms[1].

But on further observation it turned out that the idea of orientation does not apply in most of the reactions to these stimuli, so that they are not tropisms. It was in bringing out this fact: in pushing back the tropism concept from the fields whose possession it had usurped, that most of the criticism of the tropism theory arose. Happily, this condition of affairs has almost passed away; the staunchest defenders of tropisms no longer apply the concept to these fields over which controversy so long raged. This will be apparent to the reader of the recent works of Loeb[2] and of Bohn[3].

Accepting orientation as an essential feature of the tropism, and examining the part played by tropisms in the behavior of given organisms, we find, if we select the unicellular infusoria for a concrete case, that the matter works out about as follows : Tropisms are seen in the reactions to light coming from one side; to gravity; to the constant electric current, to centrifugal force, and to water currents. Of these, only the reactions to light and to gravity play any considerable part in the normal life of the organisms. A very large proportion of the infusoria do not react to light, so that the reaction to gravity remains in these as the only tropism which plays any considerable part (and that a minor one) in their life. On the other hand, the reactions to chemicals (including all food reactions), to heat and cold, to all mechanical stimuli, to osmotic pressure, to unequal illumination, — these are not tropisms, for no orientation is involved in them. These reactions compose by far the larger part of the behavior of the organisms; doubtless ninety-five per cent. or more.

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[1] For typical examples: The theory of tropisms was given for reactions to chemicals by LOEB (Pflüger's Archiv, Bd 66, 1897, p. 442), and applied in a concrete case to infusoria by GARREY (Amer. Journ. Physiol., vol. 3, 1900, pp. 291-315). The reaction to contact with solids Loeb tells us in 1900 is « only another instance of a simple tropism » (*Comparative Physiology of the Brain*, p. 184). More recently LOEB recognizes that this is not a tropism (*Dynamics of Living Matter*, 1906, p. 156). MENDELSSOHN (Pflüger's Archiv. Bd 60, 1895, and Journ. de Physiol. et de Pathol. Gén. t. 4, 1902) applies the theory in detail to the reactions to heat and cold, in infusoria. It would be easy to multiply instances of this sort. BOHN in 1906 went so far as to say that at a certain time the word tropism « était devenu l'explication nécessaire et suffisante dans tous les cas. » (L'Année Psychologique, t. 12, 1906, p. 143).

[2] *Dynamics of Living Matter*, 1906, Lecture VIII.

[3] *La Naissance de l'Intelligence*, 1909.

On this classification of behavior I take it that there would be no disagreement. If the reactions which are not tropisms be classified as to certain features of the nature of the stimulus causing them, they are what I have called « reactions to changes » in the environmental conditions[1]: what Loeb[2] calls *Unterschiedsempfindlichkeit*, what Bohn[3] calls « sensibilité différentielle[4] ».

On this matter, then, the objects of the criticisms of the theories of tropism have in the progress of investigation been fully accomplished. The complexity and variety of behavior in lower organisms; the great number of factors, internal and external, on which it depends; its modifiability and regulatory character, these things are now fully recognized; there is no further danger of domination of the field by a one-sided concept. The best evidence of this is the recent book by Dr Bohn[5], which, in its impassioned defense of the tropism concept, narrowly defines and limits its use and insists upon the great importance of other factors in behavior. With the main features of Bohn's procedure in this matter I am in hearty general agreement, in spite of his picturesque denunciation of myself as the Great Adversary.

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[1] For a full discussion of these, see Chapter XVII and many other parts of my book, *The Behavior of Lower Organisms* (1906); also my paper on *The Behavior of Paramecium* (Journ. Comp. Neurol. and Psychol., vol. XIV, 1904, especially pages 464-468).

[2] Loeb, Pflüger's Archiv, vol. LIV, 1893, p. 81; *Dynamics of Living Matter*, 1906, pp. 135-137; Journ. Exp. Zool., vol. IV, 1907, pp. 151-156.

[3] Bohn, *La Naissance de l'Intelligence*, 1909. (Bohn makes repeatedly the extraordinarily incorrect statement that I had not recognized this as a cause of reaction, when as a matter of fact it has formed the fundamental point in my presentation of the causes of reaction, and I have devoted perhaps hundreds of pages to expounding the matter. Bohn has merely introduced a new term. See, for correction of many of Bohn's erroneous statements concerning my work, a review in a forthcoming number of the American Naturalist.)

[4] I do not consider this by any means a satisfactory classification of behavior. If under the stimulus of a change in the intensity of a certain environmental agent, one organism climbs a tree, another jerks its tail, while a third writes a book, it is hardly adequate to merely classify all three as « reactions to changes » or as « sensibilité différentielle ». Similar differences are found in the reactions of diverse lower organisms to such changes in the conditions. *What the organism does* furnishes a better basis for classification; it is such a basis that I have employed in my own work. But this is aside from our present purposes. The distinction between such reactions and the tropisms is generally recognized.

[5] *L. c.*

It is clear therefore that agreement prevails over a wider field than is sometimes supposed. We all agree that there exist in behavior phenomena for which the word tropism is an appropriate name. We agree further, I judge, that a large share of behavior in lower organisms is made up of phenomena that are *not* tropisms. I believe further that there is agreement in the main on the general principles of interpretation. Loeb says in a recent paper, with regard to the tropism concept « My aim was to analyze the behavior of animals from a chemico-physical point of view and substitute the methods of modern science for the anthropomorphism of the metaphysician[1] ». With this aim we doubtless all heartily agree, and the indebtedness of biology to Loeb for keeping this ideal to the front cannot be too strongly urged. But Loeb goes on to say that « In this attempt it made no difference to me whether the elementary components of the complex « Animal Behavior » were found to be of the type » of the tropisms or of Unterschiedsempfindlichkeit « or of any other definite function ». Now, it so happens that there are certain investigators to whom this *does* make a difference; certain investigators that are interested in the actual facts of behavior; that wish to know what the elementary factors are, or whether we have really reached these elementary factors yet. It was entirely with regard to these questions of fact that criticism of the tropism theories arose. The question was as to whether a particular analysis as made is really adequate or not. My own aim was to bring out, as the facts seemed to require, the complexity, modifiability and regulatory character of behavior, and its refusal to fit any simple and uniform schemata. There is nothing in this aim inconsistent with ultimate physico-chemical analysis; the question is merely whether the problem is simple, easy, and practically solved at the present time; or complex, difficult, and hardly more than superficially touched at the present time. The latter I believe to be the case.

The Unsettled Question Regarding Tropisms.

We agree then I believe that much of behavior is not tropism. There remains a question relating to the tropisms themselves, taking the tropism as narrowly limited to reactions in which orientation is a prominent feature. This question is :

Is the tropism a well-defined, simple, uniform, elementary phenomenon? Or is it a collective concept, comprising under a single term phenomena that agree in certain respects, but differ widely in others, and including reactions of a high degree of complexity?

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[1] Journ. Exper. Zool., vol. IV, 1907, p. 152.

On this question there is still difference of opinion, some investigators holding the former view, some the latter. The best way to examine the matter will be to take up in series the main points that have been set forth as characteristic of the tropisms, and as distinguishing them from other features of behavior. I shall discuss the matter mainly with relation to unicellular organisms, since this is the field with which I am most familiar, but I shall not exclude facts derived from the study of other groups.

The Doctrine of the Uniformity of Tropisms.

There naturally arose, in the early stages of investigation, before the facts were well worked out, a tendency to assume that all tropisms are alike, — not alone in the fact of orientation, but in other respects; in the determining conditions, and in the way the organisms react to these conditions. The tropism was considered a uniform, homogeneous phenomenon, following in all cases the same laws[1]. This, in my opinion, has shown itself to be a mistake. Let us examine the facts on this matter.

In considering whether the tropisms are identical in all cases, we must ask: (A) Are the reactions of the organisms the same in all cases? (B) Is the effect of the external agent on the organism the same in all cases? (C) Is the nature of the stimulating agent the same in all cases?

A. — Are the reactions of the organisms the same in all tropisms? To anyone familiar with the multifarious activities of the lower organisms, this question needs only to be asked in order to be answered in the negative. There are several distinct points to be made here:

(1) The action of the organisms in reaching the position of orientation differs in different cases:

(*a*) In *different* organisms the oriented position is reached in very different ways, depending on the « action system », or characteristic set of usual activities of the animal in question. In becoming oriented to light the infusoria, such as Stentor and Euglena, go through complicated spiral movements, turning always toward a certain structurally marked part of the body, while the planarian worm, the sea anemone, or the starfish does nothing of the sort, but turns directly to (or from) the source of light.

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[1] For example « These tropisms are identical for animals and plants. » LOEB, *Comparative Physiology of the Brain*, 1900, p. 7. « It was possible to show that the heliotropism of animals agreed in every point with that of plants. » (*Ibid.*, p. 181).

(*b*) More important still, even the *same* organism, in different cases, — different « tropisms », — reaches the position of orientation in very different ways. This is a fact of capital significance, which needs to be clearly appreciated. It is commonly said that in the tropisms the organism turns *as directly as possible* into the oriented position (a fact that is often supposed to imply a particularly simple and elemental character in the tropism). This, as I shall show, is *not* the case, — accepting as tropisms reactions which are characterized as such by the strongest advocates of the importance of the tropism concept. In the infusoria there are two strongly marked orienting reactions that are universally recognized as tropisms, — phototropism and galvanotropism. *The action of the organisms in attaining the oriented position is entirely different in the two cases.* The facts here are perfectly well known. In galvanotropism the organism usually does turn as directly as possible into the position of orientation. It turns directly toward the cathode (or anode as the case may be), — whatever the position of its body when the electric current comes into action.

But in phototropism the position of orientation is attained *by the same organisms* in an entirely different manner. The organisms do not turn as directly as possible into the oriented position. They reach the position of orientation by a complex spiral course, always turning toward the aboral side, never toward the opposite side[1]. The method of action is like that which they show in their other reactions, that are not tropisms.

Thus phototropism and galvanotropism are in the infusoria quite different phenomena, so far as the action of the organisms in concerned. To say that they are the same is simply to leave the facts out of account.

(2) A second point that needs to be realized in this connection is that the action, the position, and the path of the organism, after orientation is complete, differ in different cases. In the infusoria the action of the motor organs is entirely different in the orientation of galvanotropism from what we find in the orientation of phototropism. In galvanotropism the cilia directed toward one pole (usually the cathode) are reversed;

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[1] Yet it is not impossible for them to turn toward the opposite side, for in galvanotropism they do so.

in phototropism nothing of the sort occurs. Again, in many animals such a position is maintained that the two sides are symmetrically affected by the stimulating agent and the path followed is such as to maintain this position. This has even been taken by many authors as a criterion and essential feature of the tropism[1]. But in other organisms nothing of the sort is found. In Paramecium, Stentor, Euglena, and the ciliate infusoria in general, and in Rotifera, the path followed in the tropism is a spiral, and the organism continually rolls over and over on its long axis. It would be difficult to imagine a path differing more from that of the starfish or of the crustacean. Certain other cases of this character we shall mention later.

These facts certainly require consideration in connection with such statements as that the path followed in the tropisms is invariable, that it can be predicted beforehand, and the like[2]. Such predictions can be made only on the basis of a careful preliminary study of the organism and its action system; of the way it responds to the stimuli to be used; they cannot be made on the basis of a general theory of tropisms. The man who, from a knowledge of how the starfish and sea anemone behave in the orientation to light, should attempt to predict how the infusoria behave under the same conditions, would fail completely. More striking still, he who from a knowledge of how Stentor becomes oriented in galvanotropism, should predict how Stentor becomes oriented in phototropism, would likewise fail completely. *Different organisms respond in the same tropisms by different methods of action; the same organism responds in different tropisms by different methods of action.* The tropism is not a uniform, elemental thing, identical everywhere, but is a descriptive and collective term for reactions having certain common characters, but differing in other respects. This appears to me a statement of known facts.

With sufficient knowledge of the nature and history of an organism, and of its previous reactions, reliable predictions can be made as to its behavior under any given conditions. In this respect there is no difference in principle between the tropisms and all other reactions. All movements of animals are, we believe, fully determined; if we know the determining causes we can predict the movements, — in other fields as well as in the tropisms. But it is an accurate knowledge of the given organism and of the conditions, that makes possible such a prediction; a general theory of tropisms is utterly unreliable for such a purpose.

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[1] For example « Nous saisissons déjà quelques-unes des caractéristiques essentielles des tropismes; une fois que le mouvement est bien établi, *il y a constamment symétrie dans l'excitation des deux côtés du corps,* et le chemin suivi peut être tracé à l'avance en appliquant les simples considérations de la mécanique et de la physique. » BOHN, *La naissance de l'Intelligence,* 1909, p. 120.

[2] See the statement quoted from Bohn, in the foregoing note; also the following: « ce qui caractérise les tropismes, c'est la direction invariable du mouvement imposée par une force extérieure. » (BOHN, *l. c.,* p. 122.)

The action of the external agent in the tropisms is sometimes said to be *irresistible*. On this point it appears to me that Dr. Bohn is in the right. « On dit souvent que les tropismes sont des mouvements *irrésistibles*, mais ceci ne s'applique naturellement qu'aux cas où les tropismes ont une intensité suffisante pour assujettir l'animal, annihiler ses autres manifestations motrices[1]. » A parallel statement could of course be made for any cause of movement whatever, external or internal. Any cause of movement is irresistible if there is nothing to resist it.

In this connection, further, it is often said that the tropisms are actions in which the *will* of the animal plays no part[2]. As a matter of fact, we know nothing whatever about the will in these lower animals. To say whether the will is or is not present in a given reaction, of course requires that the author shall have a criterion by which he can recognize the will. Most enlightened students of the subject recognize that we have no such criterion, and that such matters as will have no place in the definition of an objective phenomenon. To argue either for or against the will in such matters is placing one's self on a purely anthropomorphic basis.

B. — We have seen that the reactions of the organisms differ, in the tropisms, in different cases. How does it stand with the assumption that in all tropisms the action of the external agent on the organism is the same?

(1) The same facts which we have set forth in demonstrating the varied reactions of the organism are pertinent in answering this question. In galvanotropism the effect of the external agent is, in the infusoria, to cause reversal of the cilia on a strictly localized part of the organism. In phototropism and geotropism no such action occurs. (In reactions to chemicals, heat and cold, mechanical stimuli, osmotic stimuli, variations in illumination, — it likewise does not occur, — but these reactions are not tropisms.) Just what does occur is in these cases well

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[1] Bohn, *l. c.*, p. 132.

[2] « Nous donnerons donc le nom de tropismes à des mouvements où la volonté et les sentiments de l'animal ne sont pour rien. » (Bohn, *l. c.*, p. 117.)

known. *The effect of the external agent is visibly and undeniably different in different tropisms.* If we compare different organisms, this is of course even more strikingly true. In the planarian the first effect of getting out of orientation to light may be to cause such a contraction on one side of the body as will turn the animal directly into orientation. In Stentor or Euglena the effect is entirely different; a motor reaction of the entire animal is produced, of such a character that its first effect may be, and often is, to carry the animal directly *away* from the position of orientation.

If then we care for the objective *facts* of behavior, these facts are that the external agent has different effects in different cases: (a) in different tropisms with the same organisms; (b) in the same tropism with different organisms.

C. — Another fundamental point often set forth as one in which the tropisms all agree, and in which they differ from other reactions, lies in the nature of the stimulus to which the organisms react. The tropisms are said to be reactions to environmental agents of constant intensity, as distinguished from reactions to *changes* in the intensity of the environmental action[1].

There is certainly no *a priori* reason for combatting this distinction. It appears most natural to hold that when an organism takes and maintains a constant position with reference to some external stimulus, such as light, it is reacting to the constant action of the stimulating agent. I have no desire to maintain that this view may not hold for many organisms. But we are interested in the *facts* of behavior, as revealed by experimental analysis, and particularly in the question whether all tropisms are alike in this and other respects. As a matter of fact, the most careful and detailed analyses ever made of the typical tropism called phototropism, in the unicellular infusoria, seem to show clearly that this is not really a reaction to environmental action of constant intensity, but that, on the contrary, it is due to a series of reactions to changes in the action of the environment on the organism. I made a most thorough experimental analysis of the reaction to light of the ciliate infusorian *Stentor,* and of the flagellate *Euglena,* going much more minutely into the matter than has ever been attempted by any writer save Mast, who confirms my results. Mast made a similar and even more precise analysis for the colonial infusorian *Volvox.*

In these cases it was demonstrated: (1) that the organisms react most delicately, in characteristic ways, to *changes* in the action of the environmental agent (light) on them; (2) that such changes in environmental action are at work on the organism while it is becoming oriented to light; (3) that it reacts to these changes just as it reacts to such changes under other circumstances, where no orientation is produced; (4) that under the conditions presented by light coming from one side these reactions to changes inevitably cause the animal to become oriented and keep it oriented; and (5) that there is no evidence of any other method of action in producing the orientation. The orientation is then fully and satisfactorily accounted for by reactions to changes in environmental action (which must inevitably produce the observed effect), and there is no evidence that any other factor is at work.

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[1] See LOEB, *Concerning the Theory of Tropisms.* Journ. Exper. Zool., vol. 4, 1907.

At the risk of being misinterpreted, I shall attempt by comparing one of these organisms with ourselves to make clear the way in which experimental analysis shows the reactions to occur. As in ourselves, the part of these organisms most sensitive to light is at one side of the anterior end; this has been fully demonstrated[1]. This sensitive part may then be compared in position as well as in sensitiveness to our own eyes. Now, it is found, in Euglena, for example, that shading this sensitive « eye » causes the organism to react in a peculiar way; it causes a swinging of the body as one can swing his arm from the shoulder. It is as if shading the eyes caused one of us to sway about, swinging the head in a wide circle. Thus the body points successively in many different directions. This reaction is produced in the infusorian Euglena when the intensity of the light is decreased in any way, as by letting a shadow fall on the creature.

Now these organisms as they move forward continually revolve, like a screw boring its way through the water. This motion is a part of the normal locomotion, and is not due to stimulation. It is as if a man while swimming should continually revolve like a screw. Now, observe what must happen when the light coming from one side falls upon such a revolving swimmer. The sensitive « eye » is on one side of the head. As the head revolves, the « eye » is of course first turned toward the light, then away from it. That is, the « eye » is first subjected to light, then to shade. Now such a change, however brought about, always (as we have before noted) causes the organism to react in a peculiar way, — to wave about in the water, pointing its head first in one direction then in another. Evidently some of these movements tend to bring the animal *in line with*

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[1] An extensive paper on this matter by MAST is about to appear.

the light, while others do not. As long as it is not in line with the light, the changes from light to shade are bound to continue, so the organism is bound to continue waving about; this is what happens. But when some of these waving movements bring the body in line with the light[1], the rolling over and over does *not* cause alternations of light and shade, as will readily be seen by comparison with ourselves. If the sun were shining down directly on the top of one's head, it would cause no change in the illumination of the eyes, if one should turn around to north, south, east or west. Since then there are in this position no alternations of light and shade, there is nothing to cause a reaction in the infusorian, and it remains « oriented », with head toward the light. But if in any way it gets out of line with the light, again the alternations of light and shade must begin, bringing the organism back into line as before. Every detail of its reaction is fully accounted for in this way, and no one has ever brought forward evidence that there is any other factor involved in the matter[2].

In these cases then it appears to be demonstrated that the orientation in phototropism is due to reactions to changes in environmental action, — to alternations of light and shade on the sensitive part of the organism. Whether a sufficiently thorough analysis may not show the same condition of affairs to hold for certain higher animals I will not attempt to say. But assuming that in higher animals the tropism is, as commonly held, a reaction to a constant agent, the point of importance here is that again we find in different cases the tropism produced in fundamentally different ways. The tropism is not always a reaction to a constant stimulus; in some cases thorough analysis shows it to depend on reactions to changes in the action of the environment on the organism.

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[1] The detailed account in the original paper shows that this is inevitable. See Jennings : *Reactions to Light in Ciliates and Flagellates;* Carnegie Institution. Publ. 16, 1904.

[2] In Dr. Bohn's recent book *La Naissance de l'Intelligence*, p. 187-191, there is an extraordinary attempt to correct my account of this matter, *made in absolute ignorance of what my account is!* With my detailed experimental analysis Bohn is so unacquainted that *he does not know that I maintained that the cause of the reactions was the change in the environmental action,* although I devoted pages to expounding that point, as the essential point of my explanation. It is needless to say that Bohn gives no evidence that any other factor is involved, though he *assumes* that there is.

I regret the necessity of pointing out that Bohn's procedure in this matter is characteristic of his book. His account of my work and views is thoroughly incorrect, in a way that is most inexplicable. Readers are therefore warned against forming an idea of my work or views from Bohn's book (see the review in the American Naturalist, already mentioned).

D. — The statement is often made that the action of the external agent in causing the tropism is *direct*. What does this mean? In most cases the statement is made without further analysis, so that its deep significance is left to the imagination of the reader. Thus, Bohn says « Les tropismes, si fréquents chez les animaux inférieurs, ne sont que des réponses directes de la matière vivante vis-à-vis des influences diverses du milieu extérieur »[1]. Is there any distinction along this line between the tropisms and other aspects of behavior?

Let us try to analyse the statements that tropisms are *direct* reponses. Take for example the reaction to light. Does the statement mean that the light acts as a direct, mechanical agent, causing orientation by its *pressure*, for example? Such a theory was proposed by Radl (though even here the movement of the organism was an indirect reaction to this pressure). No one else, so far as I am aware, holds that view. Does it mean that no chemical processes intervene between the impinging of the light on the surface and the reaction of the organism? Certainly not! Such processes are fully admitted. Does it mean that the energy of the movement is all derived from the energy of the light impinging on the body? Certainly not; the movements are due to the release of internal energy. Does it mean that there is no conduction involved; that the light acts only on that part of the body on which it impinges? Such a view may have been held at one time, forming the only real basis for the idea that the response was direct, but the facts are now perfectly clear that conduction is involved, even in unicellular organisms[2]. Bohn admits, what is evidently true in higher animals, that it is quite possible that the nervous system and the organs of sense lend their aid in the tropisms[3]. In fact, it is clear that what happens in the tropism is this: the energy of light falling upon the body causes chemical changes in the body; the excitation thus arising is conducted to other parts of the body, by whatever means of conduction the organism has; this causes the release of a certain amount of the internal energy of the organism, derived from metabolism; this energy produces movements, of a sort determined by the structure and « action system » of the particular animal. I judge that no upholder of the tropism theory would present the matter otherwise. But this is precisely what happens in *any* reaction to external stimulation! There is no distinction between the tropism and other features of behavior along this line. To say that the tropism is a direct response is merely an obscure statement, with no meaning until we are informed in what particular it applies.

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[1] *La Naissance de l'Intelligence*, pp. 93-94.

[2] The idea that the stimulating agent acted merely on that part of the body on which it impinged, causing that part alone to react, thus changing the movements, was once widely held. It was this theory that I attacked, under the name of the « local action » theory. The theory seems no longer to be held, as the facts are clear that in most tropisms, as well as in other reactions even of the lowest organisms, the creature reacts as a whole. This theory gave an intelligible meaning to the statement that the action of the external agent was *direct*.

[3] *L. c.*, p. 123.

In the lowest organisms, having no nervous system, the tropisms, like all the rest of the behavior, are carried out without the aid of the nervous system. In more complex organisms, the tropisms, like the rest of the behavior, are carried out in complex ways. There is no indication that the tropism is, in this respect, any simpler or more elemental than any other feature of behavior.

We may sum up our discussion as to the uniformity and homogeneity of the tropisms as follows: The tropisms are not a homogeneous set of phenomena, for the following reasons:

(1) The reaction of the organism in becoming oriented differs in different cases: (*a*) in different organisms with the same tropism; (*b*) in the same organism with different tropisms.

(2) The action and position of the organism after it is oriented differs in different cases: (*a*) in different organisms with the same tropism; (*b*) in the same organism with different tropisms.

(3) The effect of the external agent on the organism differs in different cases.

(4) The nature of the stimulation differs in different cases.

(5) There is no indication that the stimulus acts more directly in the tropisms than in other reactions. In lower animals the reaction is comparatively simple; in higher ones it is admittedly complex. Thus the tropisms include a set of reactions showing diversities in almost every category where diversities are possible.

The fact that reactions in which orientation is a prominent feature (*tropisms*) really differ greatly among themselves has been perceived by most thorough students of behavior. It has given rise to many attempts to adopt other criteria than orientation for defining the tropism. Certain authors propose to exclude from the tropism concept all reactions save those in which the organism turns directly toward a one sided stimulus,

« Asymmetrical response to asymmetrical stimulation »[1] is made the criterion of the tropism. This form of response is of course seen in numberless other reactions which, since there is no orientation, have never been classified as tropisms, so that to adopt it as the distinctive feature of the tropism seems to result in abandoning the natural basis for the tropism concept. This restriction is not accepted by Bohn (since in his recent volume [2] he gives as examples of the tropism reactions in which this criterion is not fulfilled); so far as I can discover, it is likewise not accepted by Lœb. It would result in excluding from the tropisms most of the reactions of unicellular orgrnisms.

Another and very special criterion is set forth by Bohn for application to the case of reaction to light only. If the organism is subjected to two lights and orients with reference to one alone, the case is held not to be one of *tropisms*. In tropism, the animal will direct itself *between* the two lights, so as to produce an equal illumination of the two sides of the body[3]. This criterion would exclude many of the most famous cases that have been employed as types of the tropism, including Lœb's favorite example of the moth which flies toward the flame.

Still another criterion is proposed by Bohn in his attempt to reduce to uniformity what is in reality a mass of heterogeneous phenomena. If we suppress the sensitive surface of one side of the organism (for example, one eye, in the case of phototropism), the animal must show a *rotatory* motion [4]. Application of this criterion enables the author to exclude from the tropisms certain reactions of crabs which otherwise might be called tropisms.

The effect of these various criteria is of course to limit greatly the field to which the tropism concept can be applied. Many of the phenomena that have played a large rôle as examples of the wide distribution and great importance of the tropisms are discovered not to be tropisms at all. There is not agreement among different authors as to the employment of these criteria. It appears evident that the only natural basis for the concept tropism is the fact of orientation.

The attempts to apply these various criteria, cutting out one supposedly typical tropism after another, of course makes evident the fact that under tropisms we have grouped a heterogeneous mass of phenomena, which require classification from other standpoints. As investigation progresses the unwieldy mass will unquestionably be broken into many subdivisions.

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[1] Walter, H. E., *The Reactions of Planarians to Light*, p. 153. Journ. Exp. Zool., vol. 5, 1907.

[2] *L. c.*

[3] Bohn, *l. c.*, p. 123.

[4] Bohn, *l. c.*, p. 137.

Even in the most typical tropisms, there is reason to believe that we have by no means seized upon the really essential point in the phenomenon. As this most essential point, Bohn, Loeb, and many others, set forth the fact that when oriented the animal takes a position such that its body is *symmetrically* placed with reference to the lines of force, — to light, for example.

But consider the following strange facts. Many crustacea approach or recede from the light, showing typical orientation, with the usual symmetrical position in the light; right and left sides equally illuminated, as the theory demands. But it so happens that there are certain crustacea, the fiddler crabs, that habitually walk sidewise. We subject these to the light, and they go toward it. as do their brethren. We apparently have a clear case of positive phototropism. But in going toward the light *they walk sidewise,* as usual. That is, they do not orient, taking such a position that the body is symmetrically placed in the rays of light; on the contrary, the body is as unsymmetrically placed as possible [1].

Again, consider the phototropism of the larva of the mosquito *Corethra*, as recently described by Harper [2]. This animal moves toward the light by a series of jerks of its body from side to side. In so doing, it sometimes gets into a position of orientation, the two sides being then equally illuminated, as the theory of tropism demands. But if it retained this oriented position, it would not progress toward the light. It does *not retain the position of orientation*, but jerks out of it again, — thus making progress toward the light. It seems clear therefore that the retaining of the position of orientation is not the essential point in the reaction.

Such cases show us the inadequacy and superficiality of our present formulations and explanations of the behavior of animals. When most animals go toward a source of light, their usual method of locomotion requires them to bring the body into a symmetrical position with reference to the rays of light. But when we find an animal whose usual method of locomotion does *not* require the symmetrical position, — then this animal goes peacefully toward the light *without taking the symmetrical position!*

In these cases nature has performed for us an analytical experiment, demonstrating that when we seize upon the position of symmetry as the essential point in these reactions, we have by no means gotten to the bottom of the matter. The symmetrical position is an incident of the reaction, not its essence.

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[1] This case has recently been described by Holmes (Jour. Comp. Neurol. & Psychol. vol. 18, 1908).

[2] Journ. Comp. Neurol. and Psychol., vol. 18, 1907.

It is of course easy to maintain the view that the symmetrical orientation is the essential point in the tropism, by arbitrarily excluding all cases which do not agree with this. Any theory can be maintained, if we leave out of account the facts.

Conclusion.

I should be inclined therefore to sum up the matter as follows: Tropism is a convenient descriptive and collective term for reactions in which orientation is a prominent feature. Such reactions form a part (a very minor part, I should say) of the behavior of many organisms. The tropism is not a uniform phenomenon; the various reactions classified under the concept show diversities in practically every possible category. It is not clear that orientation is really the essential point in the tropism.

The place taken by the tropisms will be best seen by looking at them in connection with other aspects of behavior. Different organisms differ in their behavior and reactions just as they do in their structure. The condition of affairs is just what would be expected if the theory of descent with unlimited divergence were true. Among the varied reactions of animals we distinguish as a matter of description certain great categories. There are food reactions; sexual reactions; reactions of attack and defense; reactions of locomotion, etc. Among these are reactions of orientation, which we call tropisms. As in all the other great categories, these reactions are produced in different organisms in the most varied ways. Each organism uses in the orienting reactions, as in obtaining food, whatever means it has at command. Hence these reactions, like the food reactions, are simple in the simplest organisms; complex in the highest organisms, and with every intermediate gradation in intermediate organisms. These orienting reactions present no more ground for characterization as uniform, simple, elemental, or direct, than do the reactions belonging to the other descriptive categories, such as food reactions. For the real elements of behavior we must look in an entirely different direction, toward such analyses of the complex activities of organisms as are attempted by von Uexküll and Sherrington.

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The Work of J. Von Uexkuell on the Physiology of Movements and Behavior

By H. S. JENNINGS

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Bibliographic List.

(In the discussion, reference is made to the author's papers by the use of their numbers in the following list.)

1. Ueber secundäre Zuckung. *Zeitschr. f. Biol.*, vol. 28, pp. 540-549. 1891
2. Physiologische Untersuchungen an Eledone moschata. *Zeitschr. f. Biol.*, vol. 28, pp. 550-566. 1891.
3. Physiologische Untersuchungen an Eledone moschata. II. Die Reflexe des Armes. *Zeitschr. f. Biol.*, vol. 30, pp. 179-183. 1893.
4. Physiologische Untersuchungen an Eledone moschata. III. Fortpflanzungsgeschwindigkeit der Erregung in den Nerven. *Zeitschr. f. Biol.*, vol. 30, pp. 317-327. 1893.
5. Ueber paradoxe Zuckung. *Zeitschr. f. Biol.*, vol. 30, pp. 184-186. 1893.
6. Zur Methodik der mechanischen Nervenreizung. *Zeitschr. f. Biol.*, vol. 31, pp. 148-167. 1894.
7. Physiologische Untersuchungen an Eledone moschata. IV. Zur Analyse der Functionen des Centralnervensystems. *Zeitschr. f. Biol.*, vol. 31, pp. 584-609. 1894.
8. Ueber Erschütterung und Entlastung des Nerven. *Zeitschr. f. Biol.*, vol. 32, pp. 438-445. 1895.
9. Vergleichend sinnesphysiologische Untersuchungen. I. Ueber die Nahrungsaufnahme des Katzenhais. *Zeitschr. f. Biol.*, vol. 32, pp. 548-566.
10. Ueber die Function der Poli'schen Blasen am Kauapparat der regulären Seeigel. *Mittheil. d. Zool. Stat. Neapel*, vol. 12, pp. 463-476. 1896.
11. Zur Muskel- und Nervenphysiologie von Sipunculus nudus. *Zeitschr. f. Biol.*, vol. 33, pp. 1-27. 1896.
12. Ueber Reflexe bei den Seeigeln. *Zeitschr. f. Biol.*, vol. 34, pp. 298-318. 1897.
13. Vergleichend sinnesphysiologische Untersuchungen. II. Der Schatten als Reiz für Centrostephanus longispinus. *Zeitschr. f. Biol.*, vol. 34, pp. 319-339. 1897.
14. Ueber die Bedingungen für das Eintreten der secundären Zuckung. *Zeitschr. f. Biol.*, vol. 35, pp. 183-191. 1897.
15. Entgegnung auf den Angriff des Herrn Prof. Hubert Ludwig. *Zool. Anz.*, vol. 20, pp. 36-38. 1897.

THE JOURNAL OF COMPARATIVE NEUROLOGY AND PSYCHOLOGY.—VOL. XIX, NO. 3.

16. Die Physiologie der Pedicellarien. *Zeitschr. f. Biol.*, vol. 37, pp. 324-403. 1899.
17. Der Neurokinet. *Zeitschr. f. Biol.*, vol. 38, pp. 291-299. 1899.
18. (Beer, Th., Bethe, A., und v. Uexküll, J.) Vorschläge zu einer objektivierenden Nomenklatur in der Physiologie des Nervensystems. *Biol. Centralbl.*, vol. 19, pp. 517-521. 1899. Also in *Centralbl. f. Physiol.*, vol. 13, pp. 137-141. 1899.
19. Die Physiologie des Seeigelstachels. *Zeitschr. f. Biol.*, vol. 39, pp. 73-112. 1900.
20. Die Wirkung von Licht und Schatten auf die Seeigel. *Zeitschr. f. Biol.*, vol. 40, pp. 447-476. 1900.
21. Ueber die Errichtung eines zoologischen Arbeitsplatzes in Dar es Salaam. *Zool. Anz.*, vol. 23, pp. 579-583. 1900.
22. Ueber die Stellung der vergleichenden Physiologie zur Hypothese der Tierseele. *Biol. Centralbl.*, vol. 20, pp. 497-502. 1900.
23. Die Schwimmbewegungen von Rhizostoma pulmo. *Mittheil. a. d. Zool. Stat. Neapel*, vol. 14, pp. 620-626. 1901.
24. Psychologie und Biologie in ihrer Stellung zur Tierseele. Asher und Spiro's *Ergebnisse der Physiologie*, 1 Jahrg., 2 Abth., pp. 212-233. (Reprinted with the title "Im Kampf um die Tierseele.")
25. Studien über den Tonus. I. Die biologische Bauplan von Sipunculus nudus. *Zeitschr. f. Biol.*, vol. 44, pp. 269-344. 1903.
26. Die ersten Ursachen des Rhythmus in der Tierreihe. Asher und Spiro's *Ergebnisse der Physiologie*, 3 Jahrg., 2 Abth., 11 pages. 1904.
27. Studien über den Tonus. II. Die Bewegungen der Schlangensterne. *Zeitschr. f. Biol.*, vol. 46, pp. 1-37. 1904.
28. Studien über den Tonus. III. Die Blutegel. *Zeitschr. f. Biol.*, vol. 46, pp. 372-402. 1905.
29. Leitfaden in das Studium der experimentellen Biologie der Wassertiere. 130 pp. Wiesbaden, 1905.
30. Studien über den Tonus. IV. Die Herzigel. *Zeitschr. f. Biol.*, vol. 49, pp. 307-332. 1907.
31. Studien über den Tonus. V. Die Libellen. *Zeitschr. f. Biol.*, vol. 50, pp. 168-202.
32. Die Verdichtung der Muskeln. *Zentralbl. f. Physiol.*, vol. 22, pp. 34-37. 1908.
33. Die neuen Fragen in der experimentellen Biologie. *Rivista di Scienza* "Scientia," vol. 4, No. 7, 17 pp.

Some Characteristic Dicta.

"We may quietly throw overboard the entire sum of our knowledge thus far acquired, so far as it comes from experiments on the frog's leg. The nerve-muscle preparation has misled us in almost every point" (30, p. 331).

"Furthermore all electro-physiological experiments on muscles have shown themselves to be biologically worthless, so that we may here pass them over in silence" (29, p. 28).

"The fate of biology in Europe, in spite of the efforts of excellent workers, seems to me to be sealed. * * * One need not be a prophet to predict that in a few years biology will be an American science" (29, preface).

"The question of the function of the nervous system in the animal body has aroused a strife between two sciences that must end with the annihilation of one of the two combatants,—and the champions of both sides are determined to carry the combat to the end. * * * While the comparative psychologists debated concerning the amount of sensation, memory, reflection, that one should attribute to these animals, there arose in the growing science of comparative physiology an enemy to the death of all comparative psychology" (24, pp. 212, 213).

"Before objective investigation the sensations, the memory, and thoughts of animals disappeared like fluttering forms of vapor. The iron chain of objective changes, which began with the stimulation of the sense organ and finished with the movement of the muscle, was welded together in the middle. Nowhere remained a smallest spot for the psyche of the animal. Basing itself on these incontestable facts, comparative physiology pronounced the psychological conclusions mere superstitions and denied comparative psychology the right to call itself a science" (24, p. 213).

"We stand on the eve of a scientific bankruptcy, whose consequences are as yet incalculable. Darwinism is to be stricken from the list of scientific theories" (33, p. 3).

"Concerning the origin of species we know, after fifty years of unparalleled effort and investigation, only the one thing, that it does not take place as Darwin thought it did. A positive enrichment of our knowledge has not resulted. The whole enormous intellectual labor was in vain" (31, p. 168).

"When in biology one has freed himself from the idea of development,—an idea which has at last been hunted to final death,—so that one is again in position to look upon each animal as a unity closed within itself, instead of as the last chance product of an ancestral series that has been speculated together, then form and color gain a new interest and a heightened brilliance" (30, p. 318).

"Driesch succeeded in proving that the germ cell does not possess a trace of machine-like structure, but consists of throughout equivalent parts. With that fell the dogma that the organism is only a machine. Even if life occurs in the fully organized creature in a machine-like way, the organization of a structureless germ into a complicated structure is a power *sui generis,* which is found only in living things and stands without analogy" (33, p. 9).

"It is not to be denied that the vitalists are the victors all along the line. After having put an end to Darwinism, they have seized upon the entire field of the production of animal form, and now threaten the last positions of their opponents" (33, p. 14).

"So there persists in the outer world of objects an unresolvable contradictoriness—" (29, p. 129).

INTRODUCTORY CHARACTERIZATION.

J. von Uexküll, of Heidelberg, has long been one of the most active and original workers in the physiology of behavior; his work will be found of the highest interest to all seriously concerned with these matters. It undertakes for the lower animals much the same sort of analysis that Sherrington gives us for higher ones, though with many features that are in the highest degree original. Further, an examination of his work has hardly less interest as a study in scientific ideals and method than for the concrete results attained. That he has been led to radical and iconoclastic views, and that he is not afraid to express these views with decision and picturesqueness will be evident from the quotations given above. The pessimistic impression given by the quotations taken together will not escape the reader; this pessimism appears rather an unintentional result than as characteristic of the animated and militant spirit of our author. The sweeping and perhaps ill-founded character of some of the propositions advanced should not prejudice the reader against the accuracy of the author's work in his own field; this would be a serious mistake.

INVESTIGATIONS.

Von Uexküll began as a student of the nerve-muscle preparation of the frog (1). He quickly determined to carry the study of the questions involved to the lower animals; this plan, carried out largely at the Naples Station, led to fundamental results. The work first undertaken was a study of the reflexes of the cuttle-fish (2, 3, 4, 7). This was followed by a study of certain sensory problems on the skate (9), by work on the muscle and nerve physiology of the worm Sipunculus (11, 25), and by an extensive series of thorough and fundamental papers on the nerve-muscle and sensory physiology of the sea urchin (10, 12, 13, 15, 16, 19, 20). Through these studies the author had developed the outlines of an original system of nerve-muscle physiology, having at its basis the concept of *tonus*. This system he developed farther in the series of *Studies on Tonus* (25, 27, 28, 30, 31),—a series dealing with various invertebrates, which is still in progress, and which we hope may count many numbers besides the five that have appeared. Arising in connection with his systematic series of investigations there have come from his pen many incidental contributions, including notes on special points in nerve-muscle physiology (1, 5, 6, 8, 14, 17, 32), studies of rhythm (23, 26), and discussions of fundamental scientific questions (18, 22, 24, 33). In 1905 a brief textbook (29) was published, giving an outline of the views to which he had come, with directions for practical work.

Characteristic of v. Uexküll is the intellectual working over of results at the time they are reached, so as to give a graphic and comprehensible scheme of the way processes occur. This appears in the very first studies, on the cuttle-fish (2, 3, 4, 7); they show the author's characteristic abhorrence of everything vague, and particularly his objection to psychic explanations in physiology. They contain much important detail on the physiology of muscle and nerve. The paper on the skate (9) is largely a polemic against the use of terms implying consciousness in the nerve physiology of lower animals, directed mainly against W. Nagel. The account of the skate is intended to illustrate the purely objective treatment of the facts, and is perhaps not in itself a strong example of the value of the method. In this polemic, as in later work, is seen the positive, definite character of the author's thought, with no appreciation for shadings, transitions or compromises; sharpness of concept and of distinctions, black or white, is demanded everywhere.

THE WORK ON THE SEA URCHIN.

It is in the series of papers on the sea urchin (10, 12, 13, 16, 19, 20) that the author "strikes his pace" and brings out clearly the important phenomena which form the basis for his peculiar system of concepts. The plan of investigation is to work out the structure of the muscular, nervous and skeletal systems and the way they act together to produce the characteristic behavior of the animal; to work out "the biological plan" of the sea urchin. The paper on *Reflexes in the Sea Urchin* (12) gives a general sketch of this biological plan and the main reflexes which make up the behavior; the structure of the simple nervous system, the reflexes of the pedicellariæ, the spines, the tube-feet, the teeth, are briefly described. Then followed in 1899 a thorough detailed paper on the physiology of the pedicellariæ (16), a paper which must form the point of departure for all further work on these organs. The history of our knowledge of the pedicellariæ, their structure, the different sorts, their uses and particularly the laws of their movements, are developed in great detail. In 1900 came a similarly thorough, and perhaps still more important, study of the movements of the spines (19). Special studies were made of the reactions of the spines to light and shadow (13, 20); for the purpose of obtaining species favorable for this work the author made a trip to the east coast of Africa; the outfit for this trip is described in a separate paper (21).

In general, v. Uexküll found that the various organs of the sea urchin,—each spine, each pedicellaria,—can perform a number of different acts or reflexes, and that these reflexes occur to a large degree independently of each other, so that they may occur when the organ in question is isolated on a small piece of the shell. Thus each organ is a "reflex person." Yet all the differing reflexes of these various "persons" are of such a character that they work together in a systematic way to perform the necessary functions of the animal.

They carry it about, toward food and away from danger, keep the sea urchin clean, capture prey, combat against enemies, and all together do the work of life in a competent way. This is brought about without any regulation by a "higher center," merely through the essentially independent activities of the various parts. Therefore the sea urchin is characterized as a "republic of reflexes." The apparent unity of action is due merely to the way the different actions of the different parts fit into one another, by a sort of preëstablished harmony. "It is not that the action is unified, but that the movements are ordered; that is, the setting off of the different reflexes is not the result of a common central impulse, but the separate reflex arcs are so constructed and so fitted together that the synchronous but independent setting off of the reflexes, as a result of an external stimulus, produces a definite general action of the animal, just as happens in animals in which a common center produces the actions" (16, p. 390). "When a dog runs, the animal moves its feet. When the sea urchin runs, the feet move the animal" (19, p. 73).

But the author finds that the separate reflex-persons are not absolutely independent; impulses may pass from one to the other, in such a way as to produce a unified action of all. But this is due merely to a set of nerve nets having a special arrangement; it is a matter of interconnections, not of regulation from a "higher center." The question may be raised whether this distinction does not depend on an undefined and mystical use of the term higher center. If the higher center, in accordance with the illuminating ideas of Loeb, is after all essentially a place for complex interconnections, then the difference between the sea urchin and higher animals is only one of degree.

Von Uexküll finds that the separate reflexes of the various organs are not absolutely stereotyped, but the action of each part is changed at times in certain ways, often depending on the action of neighboring parts. We have then in the sea urchin an opportunity of studying coördination in its most elementary condition, and thus perhaps of determining the fundamental nature of the phenomena involved. This animal is a sort of model, which can be taken to pieces without serious alteration of the action of the parts; yet in the animal as a whole the parts do influence one another. The author therefore took in hand a thorough study of the changes in the reflexes and the way they influence each other, undertaking to formulate and define precisely the underlying phenomena. It is here that we find the origin of the most important concepts in von Uexküll's highly original formulation of behavior. It will be worth while therefore to examine carefully a sample of the author's method of analysis; for this purpose we select certain features in the physiology of the spines.

TYPICAL EXAMPLE OF METHOD OF ANALYSIS.

It will be recalled that the rounded shell of the sea urchin is covered with long spines. Each spine is a tapering calcareous rod, with a concavity at its base, by which it articulates with a hemispherical elevation of the shell. The spine is held in position by two circles of muscles radiating from the circumference of its base to the shell. These muscles, and particularly the inner circle, are steadily pulling upon the spine, thus holding it stiffly in position. This pulling takes place without external stimulus; it is due to a certain amount of tension which forms the normal condition of the muscles and continues without any such repeated contractions or tremors as are called tetanus. That is, the muscles have a certain normal *tonus.* This *tonus* becomes the central concept in v. Uexküll's formulation of the physiology of movement. The inner layer of muscles is devoted chiefly to maintaining by its constant tension a certain position of the spine; it is an example of one of the two great types of muscular action,—the "Sperrung" or tension, as distinguished from actual contraction, involving shortening. The outer circle of muscles is more active in its changes; they shorten quickly and readily, thus moving the spine in various ways. They exemplify the other great type of muscular action,—"*Verkürzung,*"—shortening or contraction. Tension and contraction v. Uexküll shows occur quite independently of each other, and this independence, with all its theoretical and practical consequences, comes to play a very great part in the later development of v. Uexküll's views; he finds it throughout animals (see 32). The neglect of the fact that we have here two entirely different functions has, the author believes, led all nerve-muscle physiology into false paths.

The first reflex shown by the spines is as follows: When a certain spot on the body is moderately stimulated, the surrounding spines bend toward it. The muscles of the side of the spine next the point stimulated contract; that is, their tonus is increased. Thus the points of the spines are directed, for example, toward an approaching enemy.

But if the stimulus is very intense, the reaction just described is reversed; the spines bend away from the point stimulated. This result is produced by a decrease in the tonus of the muscles on the side of the spine facing the point stimulated. Thus from the same spot on the body opposite effects may be produced, depending on the strength of the stimulus. This phenomenon is called by v. Uexküll reversal of the reflex ("Reflexumkehr"); it is observed in other organs of the sea urchin and other animals, under various conditions. The author holds it to be due to some sort of apparatus in the ganglion cells; an apparatus that he calls the "tonus switch" ("Tonusschalter"). This reversal is well seen in the spines when a strong chemical stimulus affects the body. Now, a further consequence of such a powerful stimulus is seen. *After* such a stimulus, even a weak stimulus, which formerly caused the spines to bend toward the spot stimulated, now causes them to bend away. So the same stimulus on the same spot may cause two different reactions, depending on what stimulus has preceded it. This phenomenon, very common in animals, v. Uexküll calls the "switching" of the tonus ("Schaltung"); he holds it due to the same apparatus as the reversal of the reflex.

The two reactions of the spines serve, under natural conditions, certain functions. The bending toward a stimulated point serves for defense; the bending away under a strong stimulus, particularly a chemical one, preserves the spines from injury, while giving opportunity for the action of certain large poisonous pedicellariæ, which now bend their envenomed jaws toward the region attacked and seize whatever is there present.

Certain other facts in the physiology of the spines are of extreme importance. A steady tension, not violent, exercised on the muscles of the spines causes them to lose their tonus; they become limp. This effect of tension on tonus is common among animals. If then a spine is pressed steadily to one side by the fingers, or by the weight of the animal's body, the muscles on the side pressed lose their tonus. The spine, therefore, becomes loosely movable in certain directions, but not in others. On the other hand, a sudden violent increase of tension, or a mechanical jar, increases the tonus, so that the spines stand out firmly.

Now, the loss of tonus, caused in the way just described, is conducted, doubtless by the nervous network, to the neighboring spines. This conduction occurs in such a way that it is the muscles of corresponding sides of the neighboring spines that lose their tonus. (This involves complicated conditions in the nervous net; v. Uexküll holds that it shows the existence of many independent nets.) Hence when a spine is pressed toward one side, the neighboring spines likewise bend in the same direction. This v. Uexküll calls the chaining of the reflexes ("Reflexverkettung"). It shows itself (an important fact) most readily when the spine is bent toward the mouth; the other spines also bend toward the mouth.

These facts have the following result. When a spot on the body is strongly stimulated, so that the spines bend away from it, the disturbance is not limited to those in the immediate neighborhood. The spines in bending away press upon the surrounding spines, tending to bend them down. They are more easily bent toward the mouth than elsewhere, so a new set of spines bend over in that direction. They again press on the next spines, bending them in turn toward the mouth. Thus a sort of wave passes toward the mouth from the point stimulated, the spines bending in turn far over toward the mouth, then back again. The entire phenomenon v. Uexküll calls the wandering of the center of excitation ("Wanderung des Erregungsmittelpunkts").

Another most important fact shows itself. Muscles that are not in tonus are much more easily stimulated to contraction than those which are in tonus. When the muscles have their usual strong tonus, it requires a powerful stimulus to cause them to contract further. But muscles which have lost their tonus as a result of steady tension (as described above), contract readily in response to even a weak stimulus, tending thus to bend the spine toward that side on which there has been tension.

From this a number of peculiar facts result. As we have seen, a moderate stimulus at a certain point tends to cause the spines to bend toward that point. If, as a result of pressure, the muscles that face the point stimulated have lost their tonus, they respond readily; the spine at once bends toward the side stimulated. But if the spines have been pressed over in the opposite direction, so that their muscles facing the point of stimulation are in strong tonus, no effect is produced; the spines retain their position. Hence, when a spot on the body is stimulated, certain spines will respond while others will not, depending on the previous tonus of their muscles. This phenomenon v. Uexküll calls "Klinkung"; those spines which are in such a condition or position that they can respond to the stimulus are said to be "eingeklinkt"; those which are not are "ausgeklinkt." These expressions may perhaps be translated by "in circuit" and "out of circuit,"—comparing the spines with instruments in an electric circuit. This condition of affairs has great importance for the functioning of the spines in locomotion and elsewhere, and parallel conditions are found in other organisms.

A similar analysis is given by the author for the pedicellariæ, tube-feet, teeth, etc.

Thus by a close and thorough study v. Uexküll has been able to analyze and formulate a number of what have been called vaguely the varying "physiological states" of organs or organisms; such analysis is needed for all cases. By making use of the concepts of Reflexumkehr, Reflexverkettung, Wanderung des Erregungsmittelpunkts, Schaltung, Klinkung, and by observing the changes in tonus and the rules for its increase and decrease, one can explain some of the most important features in the behavior of the sea urchin under natural conditions; locomotion, negative reactions to various stimuli, defence from enemies, capture of food, etc. It is, of course, no disparagement of the value of this analysis that it does not exhaust the matter for the sea urchin. Thus, when the animal is turned on its back, its spines move in ways that would not be expected from the physiological analysis based on their other movements (19, p. 105); if they did the sea urchin would not regain its normal position. In the starfish the method of action may be changed by the formation of habits, and this is doubtless true also for the sea urchin. Thus any formulation that is complete must provide also for the laws of change of behavior; for its regulatory features. Possibly no complete formulation can ever be reached, but the most direct way to approach it is by such analysis as v. Uexküll gives.

LATER INVESTIGATIONS.

We have given this account of the spines as a type of v. Uexküll's methods of analysis; by following carefully such a concrete case the reader will get a better idea of the nature and justification of his work than by any systematic survey of the concepts to which he finally comes. Let us now follow further the development of these concepts. As we have seen, the central concept is that of tonus, and the laws of the changes of tonus are the chief object of research. To research on this matter, to studying the properties of tonus in various organisms, and to devising schemata which shall help us to understand how it acts, and hence how behavior takes place, have been devoted the later researches of v. Uexküll. He has thus far analyzed from this point of view, besides the sea urchin, the worm Sipunculus (25), the brittle-star (27), the leech (28), the heart-shaped sea urchin (30), and the dragon fly (31). In the latest contribution, on the dragon fly, v. Uexküll attempts to make provision for a modification of the machinery of behavior through the experiences of the organisms. It would manifestly be impossible to resume here these researches, filled as they are with minute and technical detail.

V. UEXKÜLL'S SYSTEM OF CONCEPTS.

A view of v. Uexküll's system of concepts can be gotten most directly from his "Guide to Experimental Biology" (29). But here one does not see the development of the ideas; the actual grounds that have given origin one after another to the peculiar concepts, so that they are likely to seem on first introduction bizarre and artificial, having little similarity to anything dealt with in orthodox physiology.

The fundamental concept is *tonus.* Just what are we to understand by this? V. Uexküll at first defines it merely as the sum of those manifestations of the life of the cell that produce effects on external things (as distinguished from the internal energy used in metabolism, etc.) (19, p. 78). As his work develops, he finds need for a more precise idea of tonus. It is defined as a "form of energy" which has the property of flowing in certain ways (20, p. 474). The concept of tonus gradually becomes more and more definite. For purposes of handling and imaging it with ease, it becomes convenient to think of tonus as a fluid, which flows through a set of tubes (the nerves). This fluid becomes at last identified with Bethe's "Fibrillensäure,"—an actual chemical, visible under the microscope (27, p. 31). But this identification is not held to uniformly.

This fluid tonus is contained in a system of tubes, the nerves. "The structure of the nervous system may then be conceived as an aggregate of peculiar vessels united one with another, which interchange and equalize each other's contents with relation both to pressure and quantity" (25, p. 305). From the nerves the tonus either passes into the muscles, or causes in them the production of a fluid with similar properties, giving rise to either tension ("Sperrung") or contraction ("Verkürzung"). In dealing with tonus, either in the nerves or the muscles, we must distinguish its *quantity* from its *pressure;* these may vary independently, so that any given quantity may have high or low pressure. On the *quantity* of tonus depends the contraction of muscles; on the *pressure,* the tension of muscles.

There are certain general laws for the movements of tonus. In simple nerve nets it always flows into muscles that are extended (causing them to contract again). This is attributed to a change in the *capacity* of the muscles; extended muscles have greater capacity than contracted ones, so in extending they suck, as it were, the tonus out of the nerves. This property gives a remarkable degree of self-regulation to the action of the nerves and muscles.

In most animals, further, the tonus shows a marked tendency to flow toward a certain part of the body,—usually the anterior end,—

so that this part responds when any part of the body is stimulated. This part to which tonus flows as water flows into a valley is denominated, with poetic feeling, the vale of tonus ("Tonustal," 25, p. 310; 29, p. 56). After the tonus has flowed into certain muscles, it is possible (in some cases at least) to capture and hold it there, by cutting the nerves leading to the muscles ("Tonusfang," 25, p. 302); the muscles then remain contracted.

During rest the fluid tonus is gradually used up and disappears; at stimulation it is newly manufactured. There exist, however, reservoirs of tonus in the nervous system, so that the lost tonus of the muscles can be replaced without new manufacture.

The nervous system then contains, besides a system of communicating tubes, reservoirs of tonus; at the same time it is an elaborate apparatus for controlling the distribution of tonus. Each muscle has somewhere in the nervous system an organ which is its "representative" (25, p. 303; 29, p. 44). The office of this representative is to see that the tonus pressure in the muscle remains sufficient to cause the tension of the muscle to correspond to the weight which it has to bear. When the pressure in the muscle is insufficient, this acts on the representative (through the nerve) causing it to increase the pressure, until this raises the tension so as to support the weight. The increased pressure is produced by the fact that the representative uses up a certain *quantity* of tonus to increase the pressure of what is left.

The author's further development of this system consists in working out in detail the structure and action of this system of tubes, reservoirs and other machinery, by which the distribution of tonus is controlled. Main tubes, feeders, reservoirs, valves, etc., are devised and represented by diagrams, till we finally get figures which resemble the plan for a dye-works or a flour mill (see for example the schema for Sipunculus, 25, Tafel 6).

The method of presentation is in general the ideal construction of an apparatus which could produce the results shown by the organisms. In this construction no attempt is made to represent apparatus that actually exists in the organism; it is merely a figure or illustration; "a mere schema in accordance with which one can group the experimentally found facts in a convenient way" (25, p. 287). "The schema of indirect investigation is not a theory at all, but merely a sign language by means of which it is possible to at once express new results in a graphic ('anschaulich') way" (27, p. 31). All emphasis is laid on making the illustration thus "anschaulich"; that is, of such a character that one can "see through it"; see how it would work as a machine works. The author makes extensive use of this "fictitious schema" (25, p. 291), basing long discussions for the greater part of entire papers on its properties. Perhaps nowhere else in biology has a figure of speech, as it were, been worked out in such tremendous detail, through a long series of papers.

Regarded thus as a figure or illustration, the author appears very successful in constructing apparatus that would produce results similar in their complication and regulatory character to the processes observed in organisms. This has necessarily been done, of course, by attributing new characteristics to the various components when required. The tonus is sometimes given the characters of a definite material fluid, and much pains is taken to account for the entire quantity; again, it may be produced or disappear as required; sometimes it is considered a form of energy; at times it shows the properties of electricity in producing effects by induction (31, p. 195); at times we are informed that the figure of a fluid quite fails (25, p. 213). When the author attempts to show how his complicated machinery may become modified in a way corresponding to the production of what are called psychologically memory images (31), clearness has to be given up, and the entire figure becomes unconvincing.

As to the value of this figurative and artificial method of presenting the results of work, opinions will, of course, differ. The point can be best discussed in connection with a review of the guiding principles and scientific ideals in the author's work; to this we now turn. It is peculiarly true in the work of v. Uexküll that the author's concrete results cannot be understood without an appreciation of the principles that have guided him. This will lead us to a consideration of his general and theoretical papers.

THEORETICAL VIEWS AND GUIDING PRINCIPLES.

Perhaps the main characteristic shown throughout v. Uexküll's work is the abhorrence of anything vague, ill-defined or mystical. In his early papers he sets forth clearly the ideal of scientific work as the discovery and presentation of what is verifiable or demonstrable. "We have to do only with processes that can be objectively demonstrated, and to write the history of these processes in an animal from the moment of stimulation to the resulting reaction" (9, p. 559). This led him at once into a polemic against authors that used psychic explanations in work on animal behavior (see 7, p. 608; 9, etc.). The circle was soon widened, and in 1902 v. Uexküll declares that a war of extermination has arisen between comparative physiology and comparative psychology, a war that spells annihilation for one of the combatants and "both are determined to carry the fight to the end" (24). His fundamental point is, of course, the fact that there is no way of observing or verifying the existence of psychic phenomena in animals, so that they cannot form a part of a strictly verifiable science; and a further postulate is that all objective processes can and should be fully presented and accounted for without bringing in anything from outside. To substitute psychological interpretations for certain steps of objective experimental analysis is vicious and destructive of consistent science. V. Uexküll's polemic papers take extreme positions and are written with much picturesqueness of statement; they are of great value for rousing to a realization of the difficulties those who need such a spur. Apparently, however, all the valuable results that would be reached by utterly destroying the unhappy comparative psychologists would be equally well attained by keeping carefully separate the two fields of work. If the experimenter never substitutes a psychological explanation for a physiological one, he may also be interested, as a separate problem, in the development of mind, without injury to his objective scientific work.

This same demand for objective verifiable results, without admixture of anything else, has led v. Uexküll to take a part, with Beer, Bethe, and others, in trying to establish a purely objective nomenclature for the processes occurring in the movements of animals (18;

see also 29). This nomenclature has philosophical value and has been used by a few authors, though its employment is by no means common. There is difficulty, as with all ideal system of nomenclature, devised before investigation is complete, in the fact that its use often implies a precise knowledge of the nature of the phenomena, when such knowledge does not exist. To give precisely the correct name to a process implies that we know fully the nature of the process.

The author's abhorrence of the vague later becomes still more accentuated in the demand (which we have noticed above in our account of his investigations) that work shall be presented always in a way that is "anschaulich"; that is, in such a way that one can see just how the processes would occur, as one sees how a machine works from knowing its structure. It is extraordinary to what an extent the author makes the attainment of this "Anschaulichkeit" the chief object of biological science; he declares it plumply to be the "most essential character of all" ("die allerwesentlichste") for the science of biology (31, p. 184). "Biology is in its essence 'Anschauung' " (33, p. 16).[1] "Only the *anschaulich* structural diagram, not proving, but *showing* the unified working together of different factors, is adequate to the requirement of bringing the life processes together into an intelligible unity without omitting life itself" (31, p. 185). It is only by grasping fully the fact that "Anschaulichkeit" is the author's ideal, that one can understand many of the peculiarities of his work.

The first far-reaching consequence of this ideal arises from the fact that that which is "anschaulich" is not always that which is verifiable. The author is therefore sometimes compelled to a choice between the two, and in his later papers he at such times deliberately chooses the "Anschaulichkeit" in preference to verifiableness. He thus falls into contradiction with his own earlier requirement that we shall deal only with what is objectively demonstrable ("objectiv nachweisbar," 9, p. 559), as well as with the procedure of other investigators to whom it is more important that scientific propositions shall be verifiable than that they shall be *anschaulich.* Let us here look in a general way at the contrast between the results reached by making "Anschaulichkeit" the ideal, and those which flow from making verifiableness the ideal.

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[1] The word *intuition*, by which "*Anschauung*" is commonly translated, certainly fails to carry to most minds the same graphic idea as the German word, so that I do not employ it.

For many investigators the object of science is to prepare a system of verifiable propositions, in order that we may know what to depend on in our conduct; "to know what is true in order to do what is right," as Huxley put it. Verifiable propositions are propositions that say "Under such and such conditions you will find such and such things to occur or exist."[2] Now, if one supplies the conditions set forth, and does not find the predicted things to occur or exist, the proposition is not verifiable, and many would therefore hold that it should be stricken from science. A large proportion of the propositions concerning machine-like structures in organisms, given by v. Uexküll, do not even profess to be verifiable. One of the main objects of investigation is to find out what particular kinds of machines are present in animals, and how these actually present machines have arisen and how they are changing. This object is incompatible with the mere assumption of fictitious machines, for the first result of investigation with this object in view is to cancel these fictitious machines. This would indeed leave our science for a time less sharply formulated, but "at a certain stage in the development of a science a degree of vagueness is what best consists with fertility,"[3] for reference of the phenomena to complete fictitious machines tends to cut off search for the real ones. When we have found out what really occurs in organisms and what machines actually exist there, then our knowledge will be as "anschaulich" as the facts warrant, no more, no less.

To the present reviewer it seems that, even for practical purposes, the author has overestimated the value of a rather gross "Anschaulichkeit." The bringing in of machine-like structures,—tubes, valves, etc.,—that confessedly do not exist, seems rather to confuse than to aid the mind. It is not possible to conclude directly from the properties of the assumed machines as to what physiological properties one will find, for the parallelism is far from complete, so one must try to keep the system of machinery separate from the system of physiological facts; there are two systems to grasp in place of one. The reader finds it difficult if not impossible to disentangle statements which the author wishes to present as verifiable facts, from statements which are a mere necessity for carrying out the figurative schema. V. Uexküll shows all through his work an astounding facility in concluding as to the structures that must be present, from the functions which he sees performed. The reader wonders whether these structures are held to actually exist, or whether they are part of the fictitious schema. The reviewer finds that for his own use it becomes necessary in reading v. Uexküll's work to ask "Now, what did the author here actually observe and demonstrate?" It then becomes necessary to transform or almost re-write a paper before the verifiable results can be disentangled from the figurative presentation. I believe that this condition of affairs has prevented the work of v. Uexküll from exercising the great influence that it deserves from its importance. Nothing would be more helpful to most readers than for the author, after putting his results together in the figurative language of his peculiar system as he has done in his Guide (29), to give us a new compendium of his experiments and results, making the test of admission that which is verifiable, or at least that which the author believes will be found verifiable. This would not involve, of course, the omission of his important concepts of "Schaltung," "Klinkung" and the like, for these are names for experimentally verifiable processes and conditions; nor would it involve the omission of general laws, as verifiable statements that apply to whole classes of objects; nor would it exclude hypotheses, presented as such, for these are propositions which the author believes will be found verifiable on further investigation. It would involve simply the omission of what the author himself recognizes as fictitious. I believe it would be found that nothing of value had been lost; that the author's important work would stand out with a clearness not before attained. Further, in the technical accounts of his investigations, it would be extremely helpful if the author would at least segregate carefully his verifiable, experimental, results from his fictitious schema, if he finds that he cannot bring himself to totally abandon the latter.

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[2] Or, put in a form which holds whatever one's theories, "when you have such and such experiences, you will have such and such other experiences."

[3] Wm. James, *Psychology*, Preface.

This demand for "Anschaulichkeit" rather than verifiableness in a scientific account is what has led to an apparent opposition between v. Uexküll's work and that of some others. Such is the case, I judge, with the differences between his work and my own. He presents his work in an "anschaulich" form that is confessedly not verifiable, while I have tried to present strictly what is verifiable, whether immediately "anschaulich" or not. The results are bound to be different in the two cases. If my work should be presented by the aid of "anschaulich" fictions, or if v. Uexküll should present his own results without these fictions, the two accounts would show a most gratifying agreement; this is especially true now that v. Uexküll has included, in his last Study (31) attempts to show how his machines could be modified by the influences which act on the organism. I have never argued against the existence of machine-like arrangements in organisms. My point was merely that these machines are not fixed and final, but that they are continually changed by the environment and by the action of the organism itself.[4] Personally I believe that even these changes occur in an essentially machine-like way.

The demand for "Anschaulichkeit" at all costs is apparently what has led the author to certain extreme views; to his separating and contrasting biology and physiology; and to his tendency to fall into vital-

[4] In his recent paper on *New Questions in Experimental Biology* (33) v. Uexküll, in presenting a graphic picture of my exposition *if carried to a logical extreme*, has attributed to me extreme views which I have never held. He says that I "denied the existence of the reflex; denied the existence of *any structure* in the central nervous system." This statement I am sure is given as part of an "anschaulich" fictitious schema, not as a statement of verifiable fact; I have made no such denial. Again he quotes me as saying that "the organism is *only* something happening," when what I said is that "The organism is something happening." The difference is like the difference between black and white. I was trying to insist upon certain facts that had been commonly left out of account,—not trying to substitute these facts for everything else known.

ism at certain junctures. Having abandoned (in favor of the construction of fictitious machines) the requirement of finding out what are the real forces at work in organisms, of finding out what machines actually do exist (as determined by the test of verification), the author finds himself in opposition to physiology, which searches precisely for the real (verifiable) forces, materials and machines of organisms. To escape this opposition, v. Uexküll renounces physiology and all its works; renounces finding out the causes of things, and calls himself a biologist only; biology he maintains has an entirely different purpose from physiology. "We distinguish two sciences of animate nature; Physiology, which arranges her materials according to causality; Biology, which arranges her materials according to purposiveness (Zweckmässigkeit)" (29, Vorwort). The purpose of biology is to work out the *plan* according to which the body is made up and acts (33, pp. 10, 11, etc.). The *materials*—the actual chemical and physical substances, properties and forces—used in realizing the plan, do not concern biology, but form the field of physiology (28, p. 376). Hence the biologist may content himself with schemata which reproduce what the organism does, even though the organism and the schema are operated by different forces acting on different materials in different arrangements. Thus "When I for example lay out the plan of structure of a worm, and in so doing use any convenient physical schema, it doesn't occur to me at all to touch upon a physical problem. One may always think of any other force as at work in the same object. I am not concerned with that. I seek only for a fitting expression in order to make the plan of the animal *anschaulich*" (28, p. 377). The biologist need not concern himself with causal questions; with physiology. "It is therefore not to be complained of if we biologists, who are asking about the functions of animals, look with much coolness at the end problems of physiology" (28, p. 377).

Renouncing then a causal study for biology, and holding that "Anschaulichkeit" or the demonstration of the production of processes in a machine-like way is the "'most essential of all" things in biological explanations, v. Uexküll naturally gets into serious difficulties when he confronts processes which he is unable to present as "anschaulich" by "searching about for a satisfactory mechanical scheme of structure" (31, p. 188). Such he feels that he finds in all developmental processes, both in development from the egg, and in the development of new features in movement and the organs of movement. "It is greatly to be regretted that we must give up the hope of finding an *anschaulich* structural schema for animal development. But there is no structure that could explain (veranschaulichen) its own production" (31, p. 185). Since, then, it is impossible to bring development under the only point of view which seems to v. Uexküll to give a satisfactory explanation, he finds it necessary to take refuge in vitalism. He is, however, under no illusions as to vitalism's being an explanation; it is a mere renunciation; "when we therefore give over the production of form to vitalism, this giving over involves a renunciation of all real understanding in this science" (31, p. 187). In his latest paper v. Uexküll counts himself, if I understand him correctly, as a vitalist so far as developmental processes go, but as a "machinalist" so far as the functioning of developed organs is concerned (33, p. 14).

If in place of making "Anschaulichkeit" the end to be reached, one takes verifiableness as his aim, a very different set of views will be reached in biology. There are many fields of exact science in which such "Anschaulichkeit" as v. Uexküll demands is not required. To understand how water is produced from oxygen and hydrogen, most chemists do not construct a fictitious machine on the plan of a flour mill or a dynamo. They merely accept the fact as a datum, in connection with other similar facts. V. Uexküll himself mentions a number of fields of science which are not "anschaulich" in character (33, p. 16), so that it seems extraordinary to found vitalism on the basis that biology is similar to other sciences in this respect! The only condition that science requires in order that accepted principles of explanation shall apply is this; that differences in resulting conditions shall always be found to be preceded by differences in foregoing conditions, so that nothing shall happen undetermined. But why oxygen and hydrogen in the proportion of one to two should give the properties of water rather than those of alcohol we do not know any more than we know why in biology one combination produces a sea urchin, another a starfish. Throughout both chemistry and biology we find unpredictable results produced by new combinations. The repeated changes shown by the development of an organism seem, as to intelligibility, quite on a par with a series of transformations due to recombinations of chemicals. If in either field the same combination under the same conditions should sometimes produce one result, sometimes another, then indeed science would be in distress, and if biology were the field in which this occurred, then the biologist might perhaps grasp at vitalism as a drowning man grasps at a straw. Our quotation from v. Uexküll (given above, p. 316), in which he holds that Driesch has shown that the germ cell "does not possess a trace of machine-like structure, but consists of throughout equivalent parts" and that it is "structureless," perhaps implies that he conceives this distressing condition to have been reached. But those who have spent years in working with the astoundingly complex machine-like structures and processes in the chromatin of the germ cell, and have considered the demonstrative evidence brought forward by Boveri, Wilson, Herbst and many others as to the distinctive functions of these various parts in development, will find the statement that the germ cell is structureless and composed of throughout equivalent parts so absolutely schematic and fictitious as to omit *all* the truth!

Taking verifiableness as our aim will likewise leave biology and physiology resting peacefully in union. We shall be interested in the plan of the organism so far as it is *verifiable;* and to work out the verifiable plan we shall be forced to consider the actual forces, materials and arrangements, not fictitious ones. Doubtless physiology has in practice become narrowed; the remedy lies in broadening it till it includes everything verifiable in the study of the processes of organisms.

Criticism of theoretical points is not a proper close for a consideration of work of such solid value as that of v. Uexküll. Though we may differ from him in theoretical ideal and in method of presentation, we must recognize the fundamental soundness of his methods of actual work. Never was a truer principle set forth for successful biological investigation than that the first requirement is "The continued and accurate observation of the living animal in its environment" (29, p. 75). And v. Uexküll has done more toward an analysis of the internal processes in the behavior of lower animals than perhaps anyone else.

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